



**Introducing Owlstone's Breath Biopsy VOC Atlas:
identification of breath VOCs with TD-GC-Orbitrap
high resolution accurate mass spectrometry**

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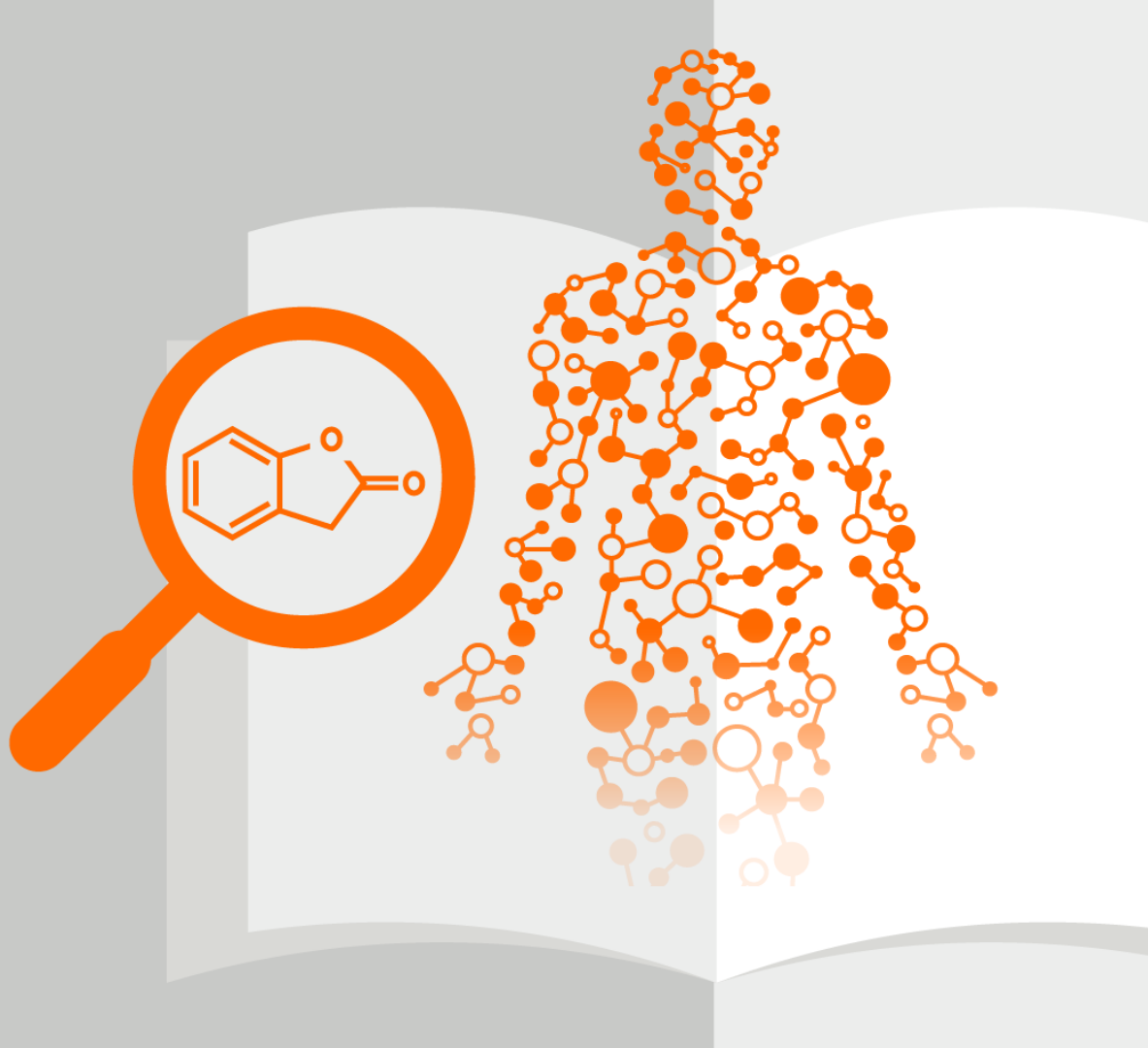
Shane Swann – Owlstone Medical Employee

owlstonemedical.com



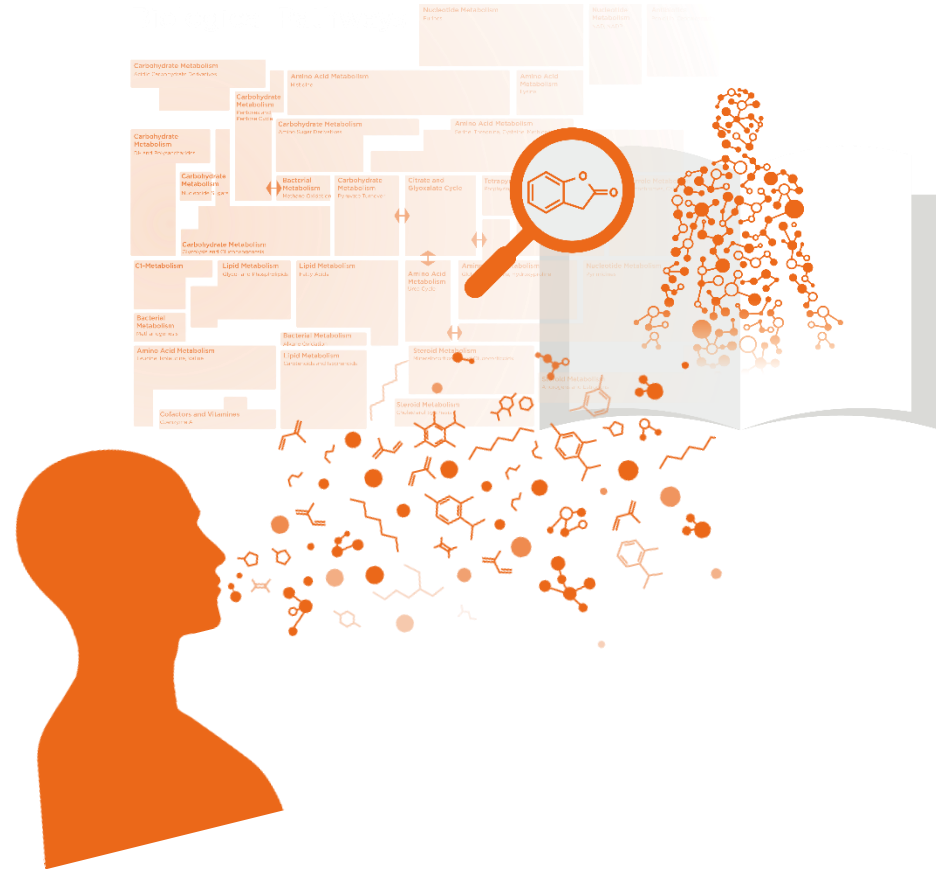
Agenda

- Why use breath?
- What is the breath Atlas?
- Creating the breath Atlas?
- How do we use the Atlas?

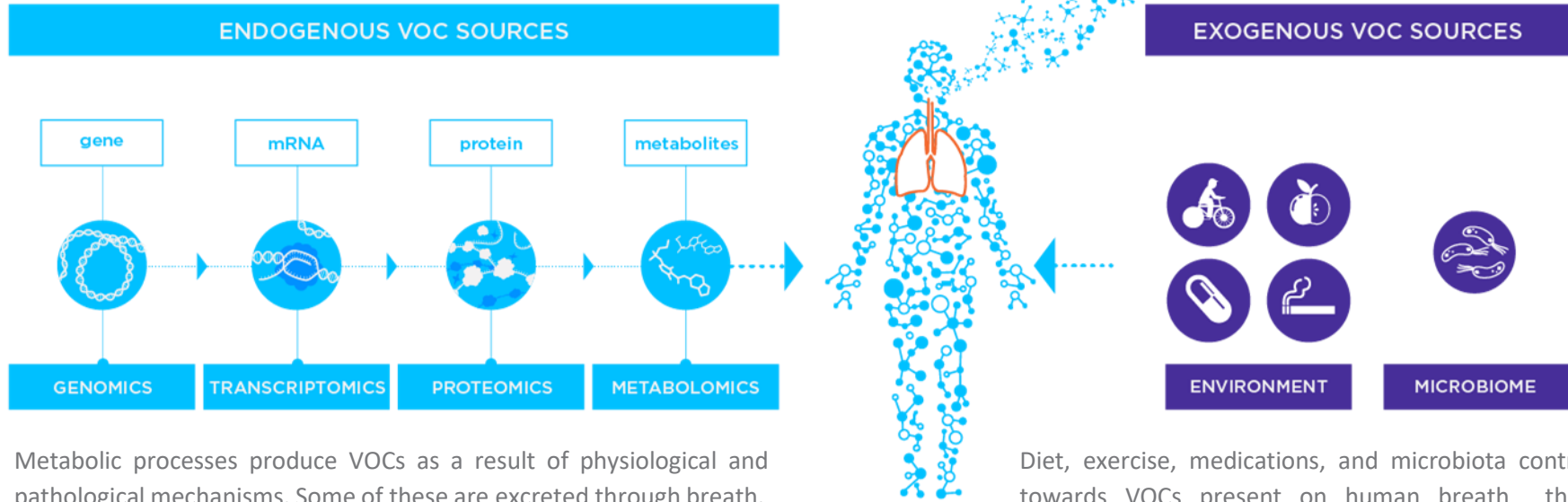


What is the breath VOC Atlas and what are its benefits?

- Owlstone's breath VOC Atlas: a growing list of VOCs found on breath in diverse healthy participants.
- Benefits: understanding biological pathways and behaviour of breath VOCs, aiding biomarker validation.
- Helps optimize processes in breath sampling platform.
- Enables targeted performance improvement of compounds during breath analysis.



VOCs on Breath are a Mix of Endogenous Metabolites and Exogenous Chemicals

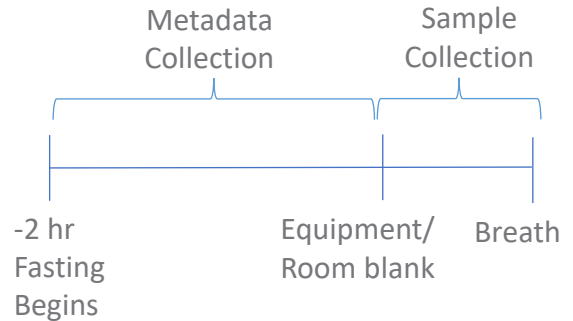


Metabolic processes produce VOCs as a result of physiological and pathological mechanisms. Some of these are excreted through breath.

Diet, exercise, medications, and microbiota contribute towards VOCs present on human breath through distribution and metabolism of external compounds. Environmental exposures and background contaminants are also present.

Study to Collect On Breath VOCs

- Breath and background samples collected during the same visit for all participants
- Questionnaire-based metadata includes:
 - Age
 - Gender
 - BMI
 - Resting BP
 - Behaviours (diet, exercise, smoking status, etc)



Mean storage time: 15 days

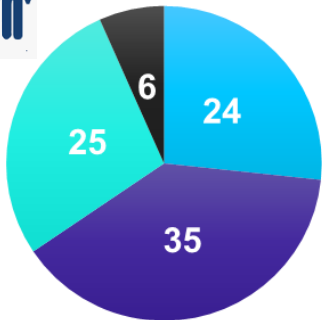
Study Cohort		
Age Group (yr)	Male (n)	Female (n)
18-30	10	14
31-50	19	18
51-70	12	15
71+	4	2

Ethnicities						
Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
64	7	9	11	1	1	1

Demographic Details

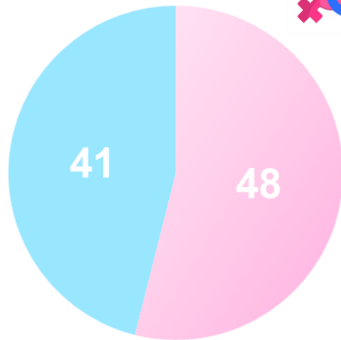


Age



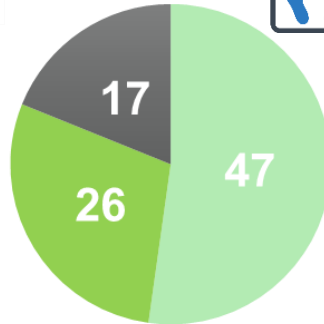
■ 18-30 ■ 31-50 ■ 51-70 ■ 71+

Sex



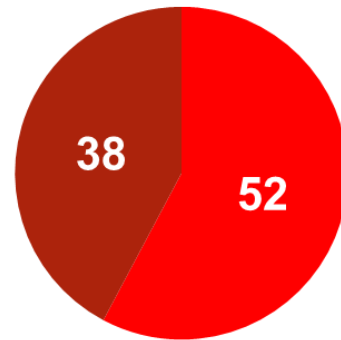
■ Female ■ Male

BMI



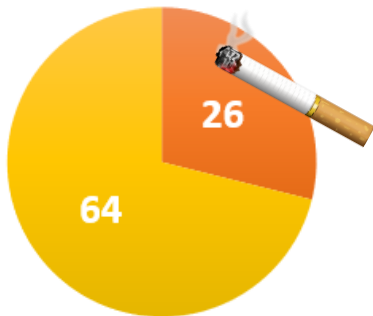
■ <25 ■ 25-30 ■ >30

Systolic blood pressure



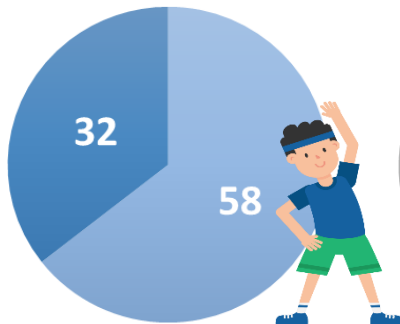
■ <130 ■ >130

Ever smoked?



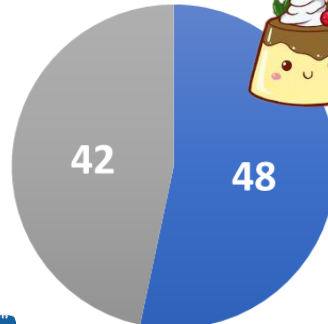
■ Yes ■ No

Exercise in last 24h?



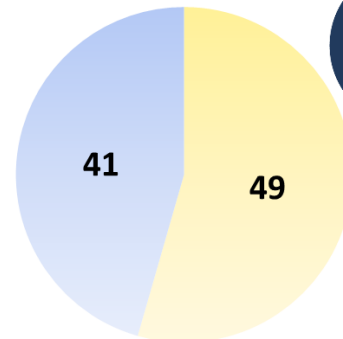
■ Yes ■ No

Eaten within 4h?



■ Yes ■ No

Time of sampling



■ Morning ■ Afternoon

The OMNI[®] platform



Breath Biopsy Collection Station enables reproducible breath sample collection and maximizes signal to noise ratio. Through ReCIVA, it collects and concentrates VOCs from large volumes of breath for high sensitivity and molecular diversity.

Collection



GC-MS analysis on high-resolution accurate mass (HRAM) Thermo Scientific[™] Q Exactive Orbitrap systems further enhances analyzable molecular diversity, and reliable identification of VOCs.

Analysis includes deconvolution, feature extraction and normalization.

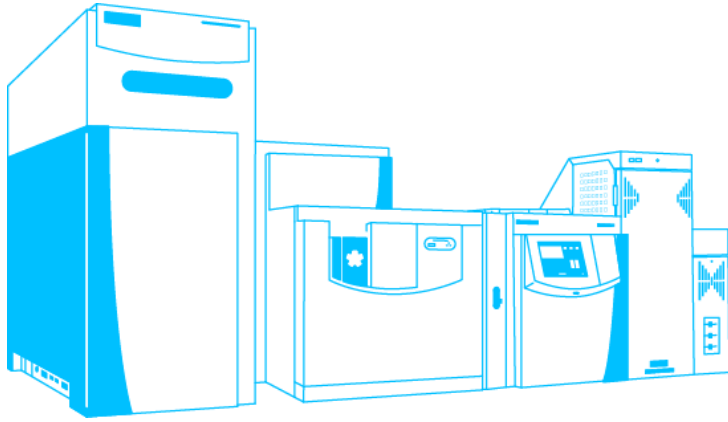
Analysis



Specialist data interpretation using NIST VOC Library and Breath Biopsy VOC library for high confidence VOC ID assignment. Reporting contains a complete feature table of scaled and normalized VOCs.

Interpretation

Breath Analysis Using TD-GC-MS



- We use **TD-GC-MS (Orbitrap)** – creating the **Gold Standard** for VOC biomarker discovery.

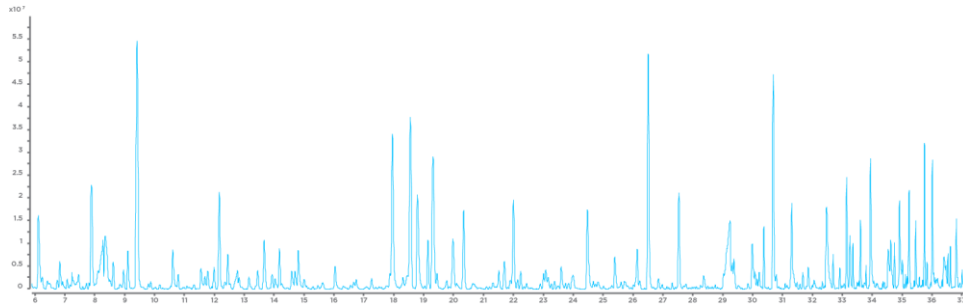
- **Column:** TG-624SiIMS 1.4 μm film x 0.25 mm ID x 30 m

- **GC Conditions:**

	Rate (°C/min)	Temp (°C)	Hold time (min)
Initial	0	37	4
1	5	150	0
2	20	300	10.9

- **MS Settings:** Full scan m/z 30 to 450

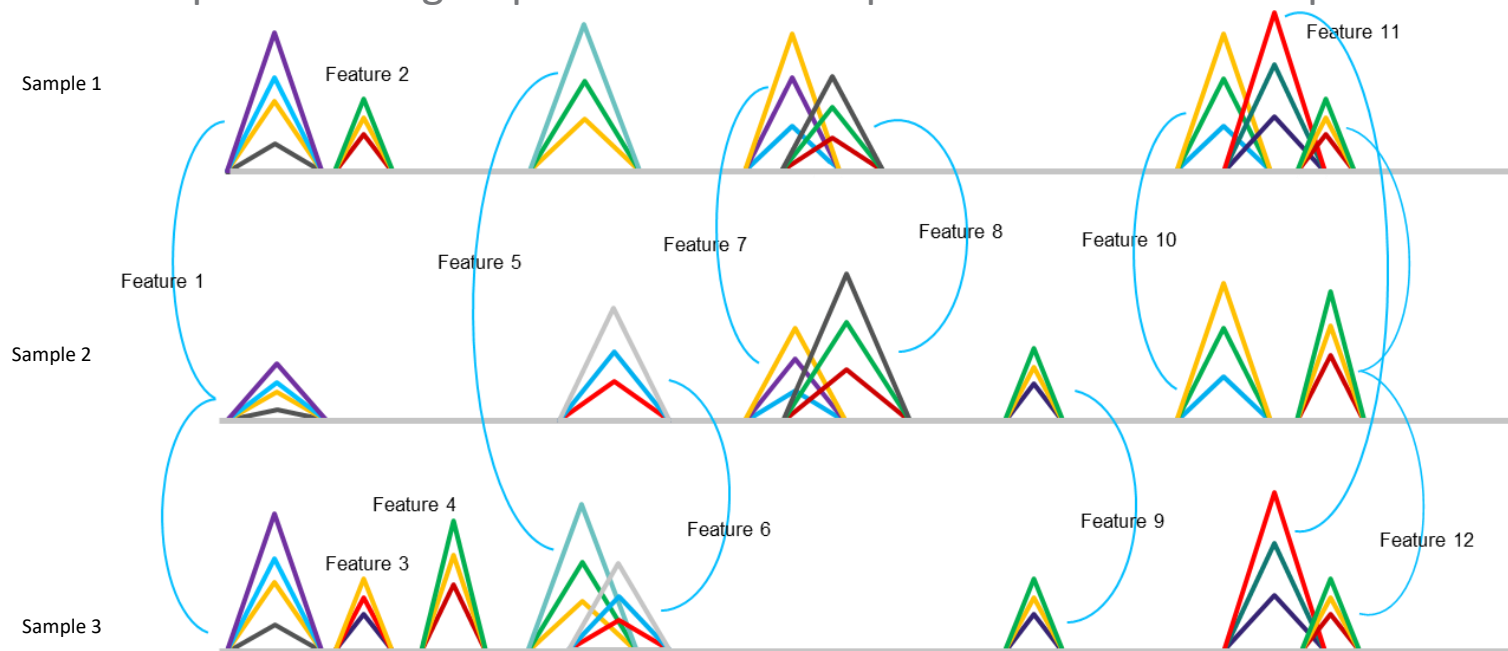
Resolution 60,000



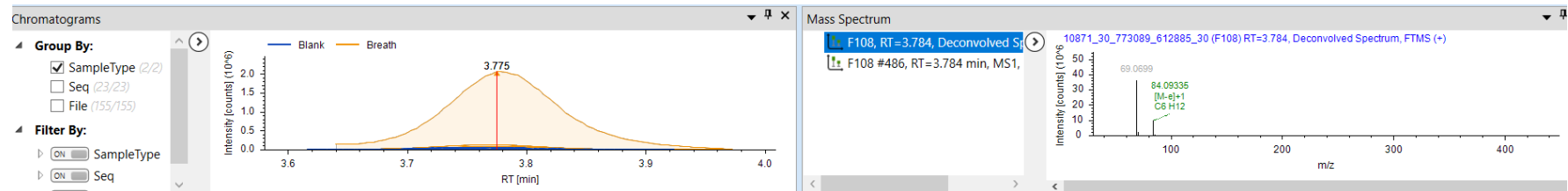
Thermo Scientific™ TRACE™ 1310 GC system connected to Q Exactive GC Orbitrap MS

Untargeted FE: VOC grouping

- Peak detection and deconvolution: fragment ions with the same peak shape and RT are grouped together to create compounds
- Compounds are grouped across all samples based on RT and spectra similarity



Extracted VOCs from Compound Discoverer



GC EI Compounds		Input Files	Study Information									
Checked	Tags	Name	Calc. MW	RT [min]	Reference m/z	Avg TIC	NIST Lib Hit Formula	NIST Theo. Mol. Mass	NIST Observed Mol. Mass	Total Score	HRF Score	SI
<input type="checkbox"/>	○○○○○	Cyclopentane	70.07828	3.743	56.05763	11315835	C5 H10	70.07770	70.07773	90.3	75.8	996
<input type="checkbox"/>	○○○○○	1-Butene, 3-methyl-	54.04697	3.745	161.98991	1213333	C5 H10	70.07770		88.1	88.9	627
<input checked="" type="checkbox"/>	●○○○○	2-Butene, 2,3-dimethyl-	84.09390	3.775	70.07326	694480	C6 H12	84.09335	84.09335	97	99.6	856

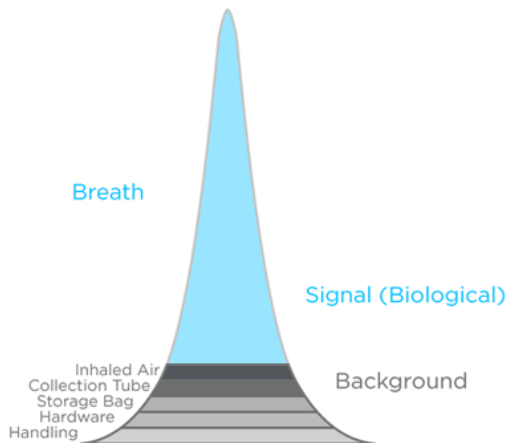
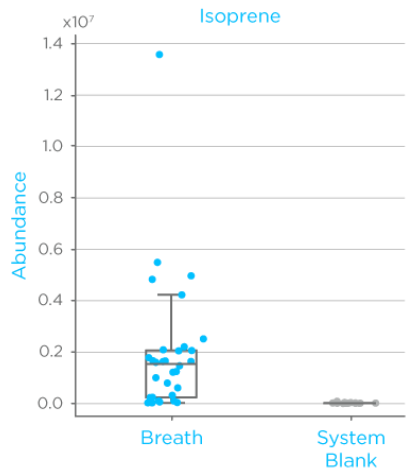
Hide Related Tables

GC EI Compounds per File **NIST Library Search Results**

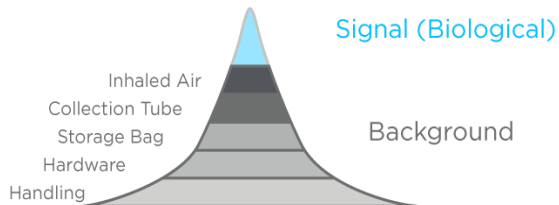
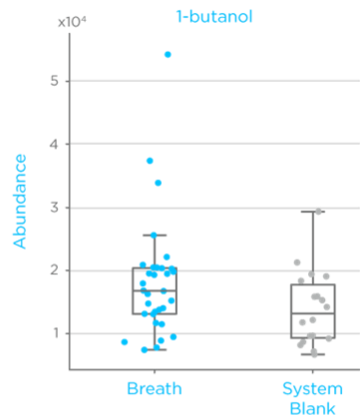
Checked	Structure	Name	CAS Num	Formula	Total Score	HRF Score	RHRF Score	SI	RSI	Elements Found[%]	Molecular Weight	Theo. Mol. Mass	Observed Mol. Mass	ΔMass [Da]	ΔMass [ppm]	M+ In List
<input checked="" type="checkbox"/>		2-Butene, 2,3-dimethyl-	563-79-1	C6 H12	97.0	99.6	99.6	856	856	100.0	84.09390	84.09335	84.09335	0.00000	0.02	Yes
<input checked="" type="checkbox"/>		2-Pentene, 3-methyl-, (E)-	616-12-6	C6 H12	96.3	99.6	99.6	822	823	100.0	84.09390	84.09335	84.09335	0.00000	0.02	Yes
<input checked="" type="checkbox"/>		2-Pentene, 3-methyl-, (E)-	616-12-6	C6 H12	96.3	99.6	99.6	821	822	100.0	84.09390	84.09335	84.09335	0.00000	0.02	Yes

Maximize On-Breath Compound Detection

ON BREATH

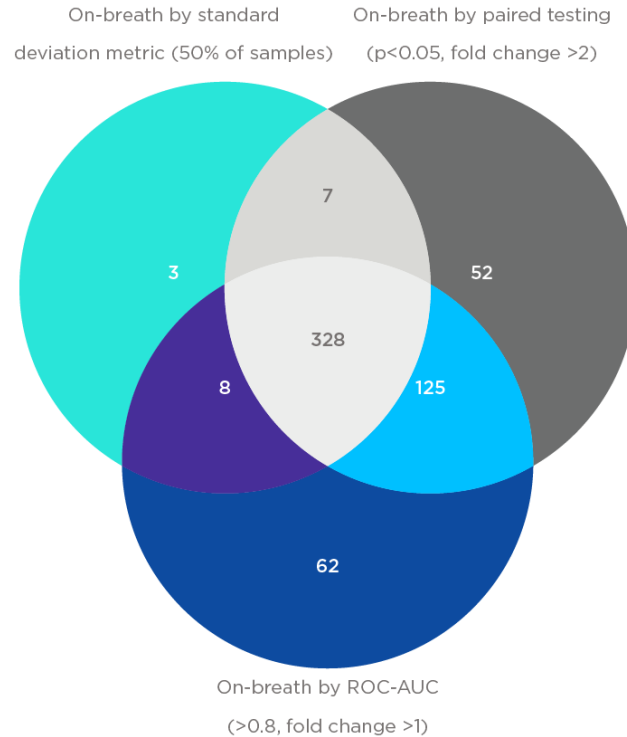


NOT ON BREATH



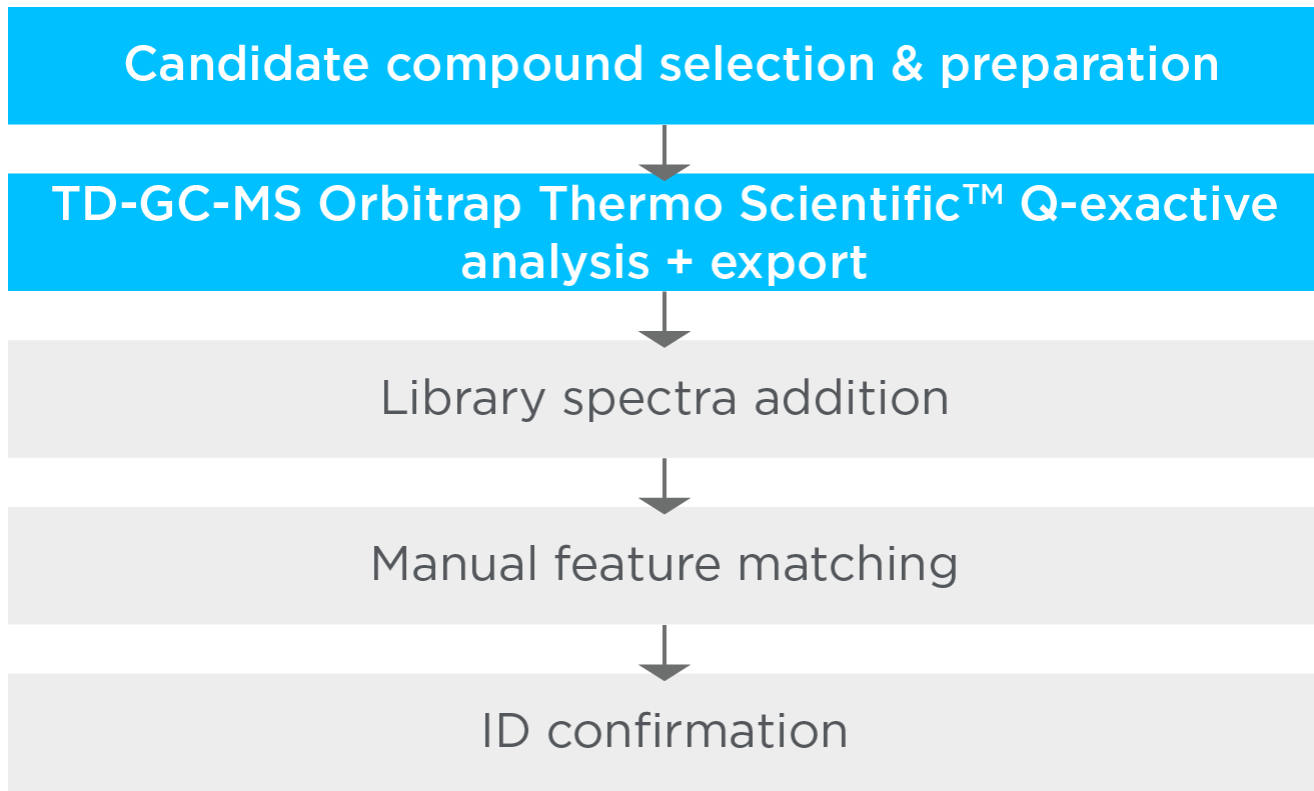
Total on-breath features

- 346 on-breath features by our "standard deviation" metric
- 585 by at least one of our 3 metrics



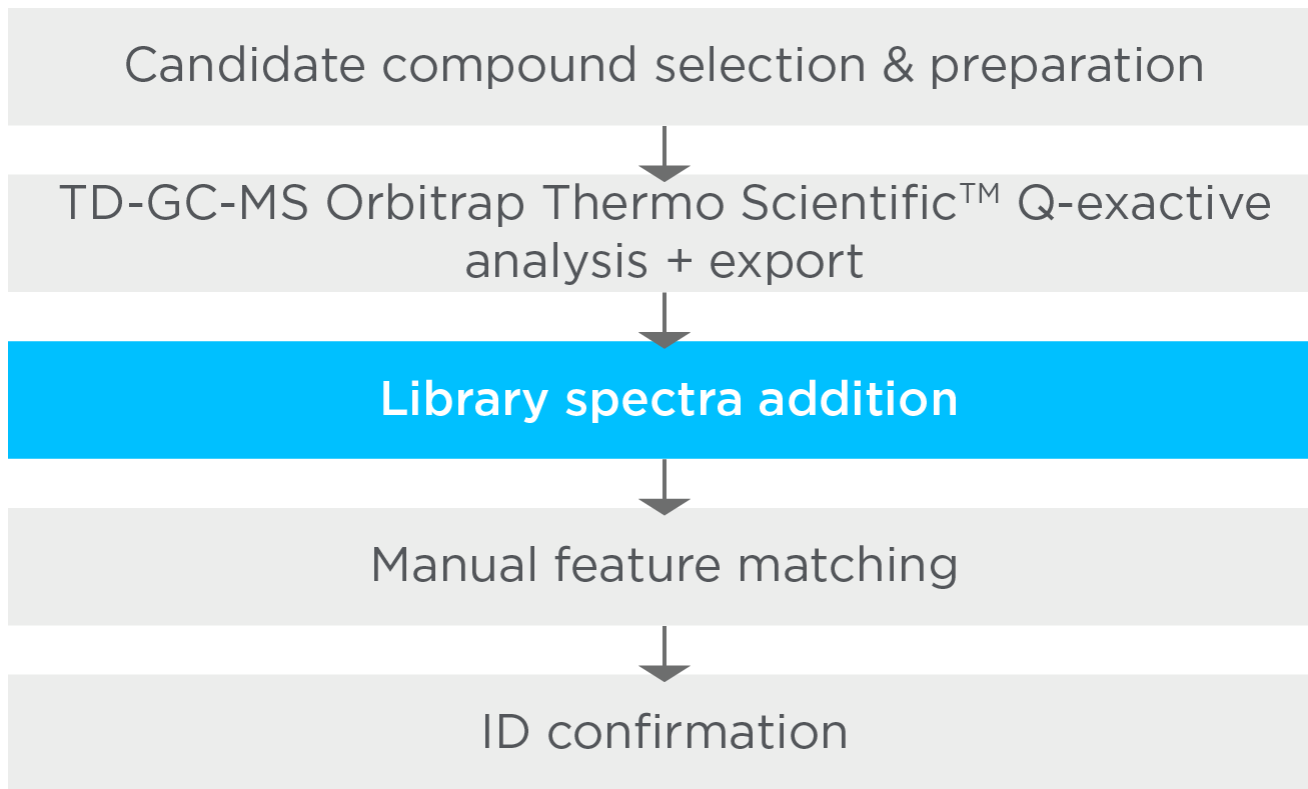
Compound ID workflow

The Compound ID workflow provides a standardized end-to-end process for the identification of on-breath compounds.

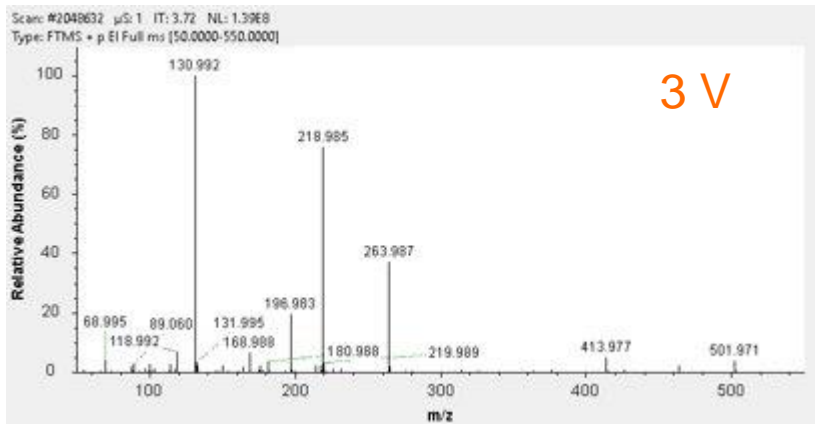
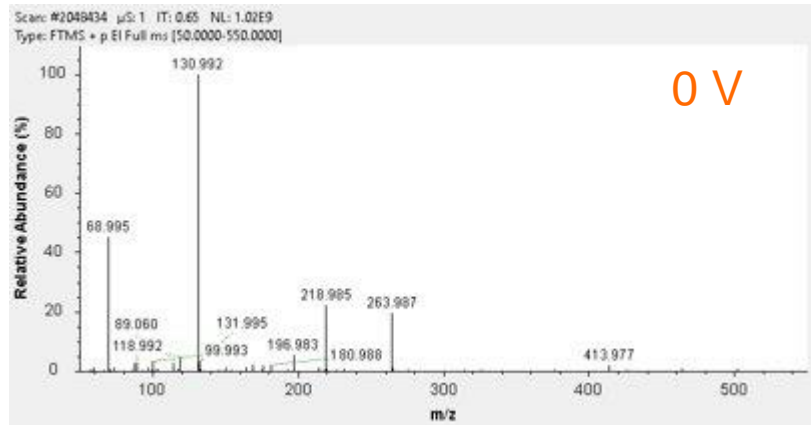
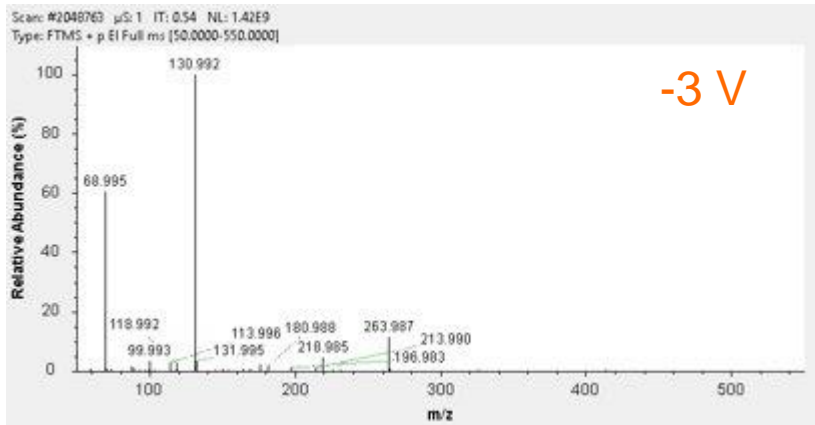


Compound ID workflow

The Compound ID workflow provides a standardized end-to-end process for the identification of on-breath compounds.



Need to Reconstruct Libraries



- Influence of C-trap on ion fragment abundancies
- Example shown for PFTBA calibrant solution for MS
- Decrease value to promote trapping of lower m/z ions
- Increase value to promote trapping of higher m/z ions

Library spectra addition

GC Decon App

File View Help

Samples: (6) FTMS - p EI Full ms [30.0000-450.0000]

Status	Sample name	Sample Id
▶	11887_3_316902_82-1_03	
▶	11887_4_773073_82-2_04	
▶	11887_5_784865_83-1_05	
▶	11887_7_772750_83-2_07	
▶	11887_8_772853_84-1_08	
▶	11887_9_664153_84-2_09	

Peak List: (376)

Component Name	RT	Ref m/z	Area	Height	TIC	C
Oxo-(5-phenylimidazo[1,2-a]...	30.918	221.084457	1092195	455269	861656	
Phthalic acid, 4-isopropylh...	30.950	163.039017	1555296	601835	886789	
4-Cyclohexene-1,2-dicarbox...	30.963	93.057465	131520	33957	162388	
Pyridine, 2-phenyl-	30.981	154.065186	3707426	1336034	3533973	
Peak@30.999	30.999	205.103622	87838	36221	167095	
Peak@31.004	31.004	204.100052	1070136	438670	1221644	
1,3-Dioxolane, 4-methyl-2-(2...	31.017	87.046577	1298198	534953	1770216	
Propane, 2,2'-jethyldenebis...	31.022	87.047272	1829064	733668	2757076	
Tetraglyme	31.031	59.049129	1181543529	430907188	1134050767	
1,1-Dioxane, 2-hydroxy-	31.036	42.046257	30059394	10482550	38116000	

Peak Identifications: (44)

Score	Matched Compound	CAS	SI	HRF Score	RSI	RHRF Score	Library
▶ 95.8	Tetraglyme	143-24-8	822	98.3138	825	98.3092	replib
95.6	Tetraglyme	143-24-8	813	98.3138	815	98.4319	mainlib
95.6	Hexaethylene glycol dimeth...	1072-40-8	812	98.3138	812	98.3128	mainlib
95.6	2,5,8,11-Tetraoxadodecane	112-49-2	814	98.3138	824	98.4165	replib
95.4	Tetraglyme	143-24-8	805	98.3138	805	98.3111	replib
95.3	2,5,8,11,14,17-Hexaoxaoct...	1191-87-3	800	98.3138	800	98.3138	mainlib
95.2	2,5,8,11-Tetraoxadodecane	112-49-2	793	98.3138	798	98.4254	mainlib
94.9	Ethanol, 2-[2-(2-methoxyeth...	112-35-6	777	98.3138	788	98.4125	feat_11870_ms...
94.9	2,5,8,11-Tetraoxadodecane	112-49-2	777	98.3138	792	98.2979	replib

Rawfile: R:\Clinical\Internal_Breath_Data\Orbtrap\ORB-03_Data\FEAS
 Acquisition date: 25/08/2022 13:59:49
 InstrumentName type: Q Exactive GC Orbtrap
 Inst ID: Exactive Series slot 148

Extracted Ions

Spectra

Active	Measured m/z	Area	Height	Fragment ID	Theo m/z	Mass error (ppm)
▶	<input checked="" type="checkbox"/>	59.049129	1181543529	430907188	C3H7O	59.04914141... 0.210164
	<input checked="" type="checkbox"/>	58.041340	333965759	121454137	C3H6O	58.04131638 0.406951
	<input checked="" type="checkbox"/>	87.044083	315436029	116529957	C4H7O2	87.04405601 0.310073
	<input checked="" type="checkbox"/>	103.075333	286605005	106693437	C5H11O2	103.07535613 0.224399
	<input checked="" type="checkbox"/>	45.033524	208872288	73739670	C2H5O	45.03349135 0.729015
	<input checked="" type="checkbox"/>	31.017879	171372709	63324326	CH3O	31.01784129 1.215750
	<input checked="" type="checkbox"/>	43.017792	145743463	51646629	C2H3O	43.01784129 1.145805
	<input checked="" type="checkbox"/>	89.059731	61275083	22726976	C4H9O2	89.05970607 0.279924
	<input checked="" type="checkbox"/>	73.028442	57791824	21095917	C3H5O2	73.02840595 0.493643
	<input checked="" type="checkbox"/>	61.028442	48602851	14890999	C3H5O	61.02840595 0.346187

Component spectrum

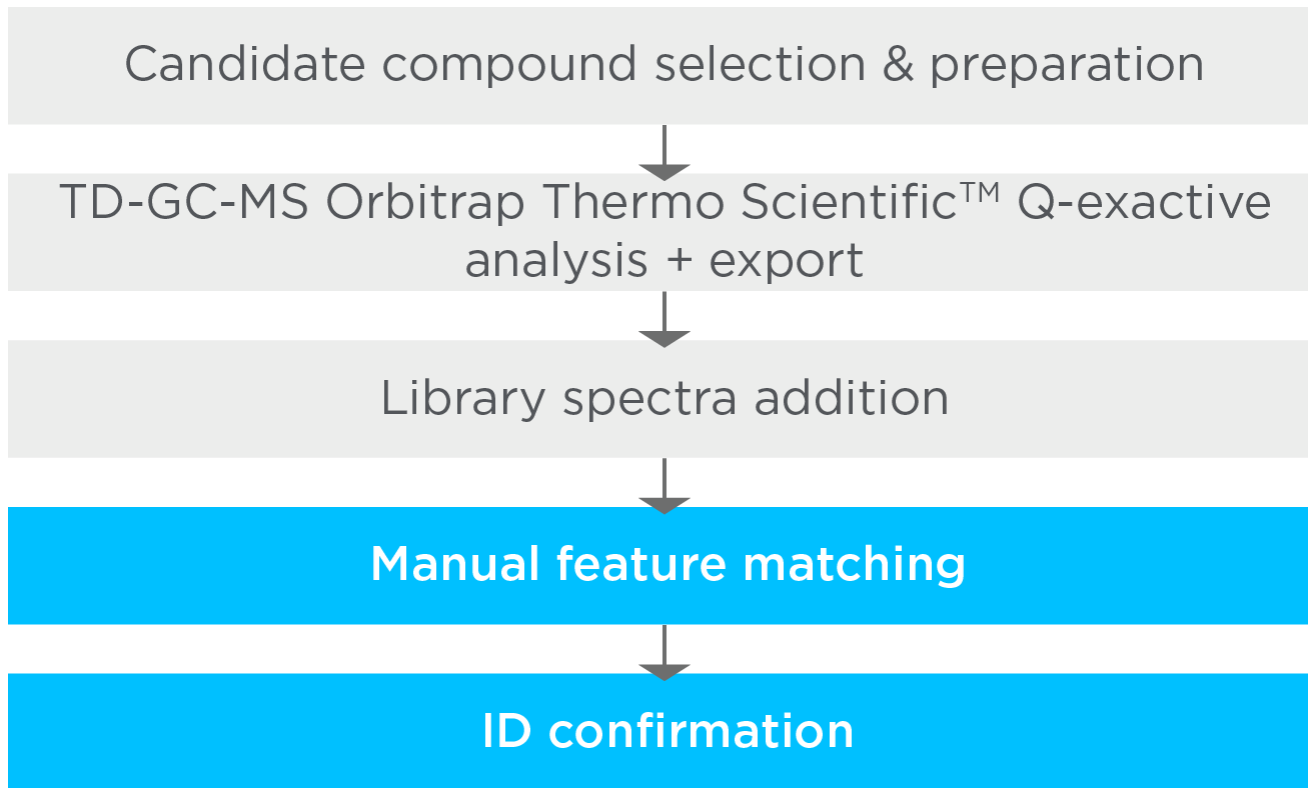
Relative abundance

20 52 85 117 150 182

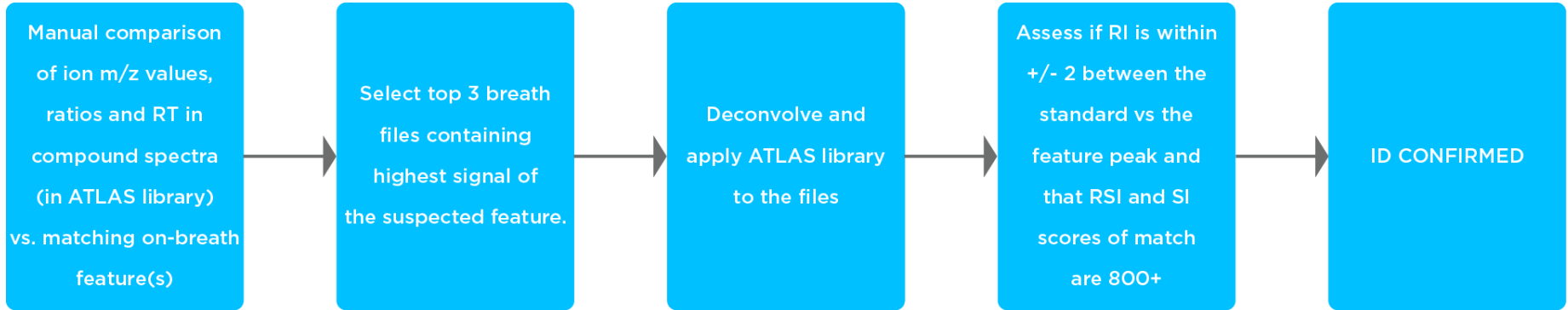
Activate Windows
 Library spectrum activate

Compound ID workflow

The Compound ID workflow provides a standardized end-to-end process for the identification of on-breath compounds.

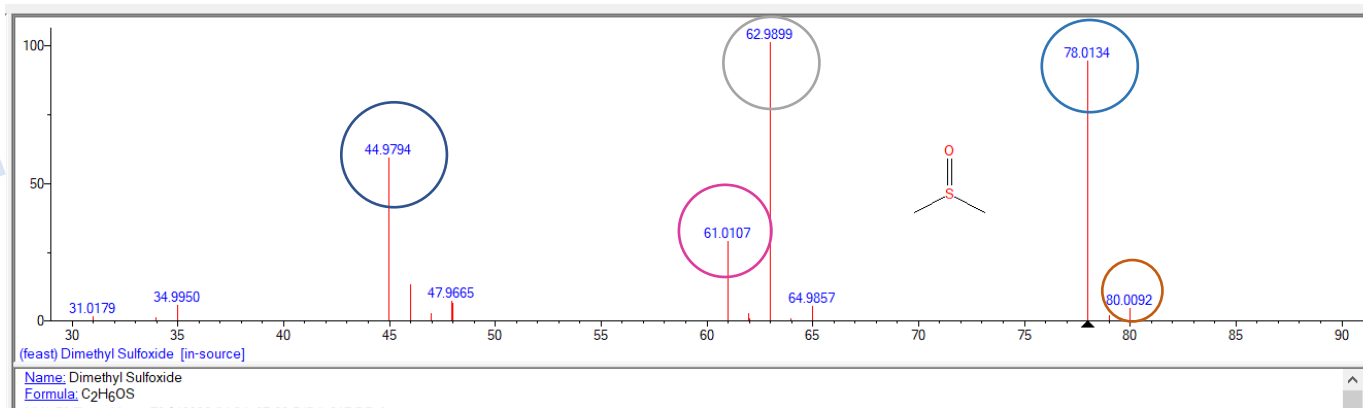


Feature matching and ID confirmation



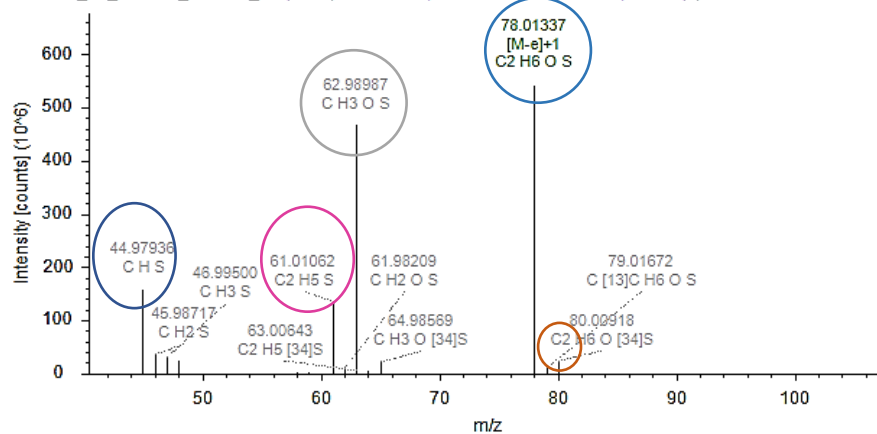
Manual feature matching process

In-house library entry
for
Dimethyl Sulfoxide

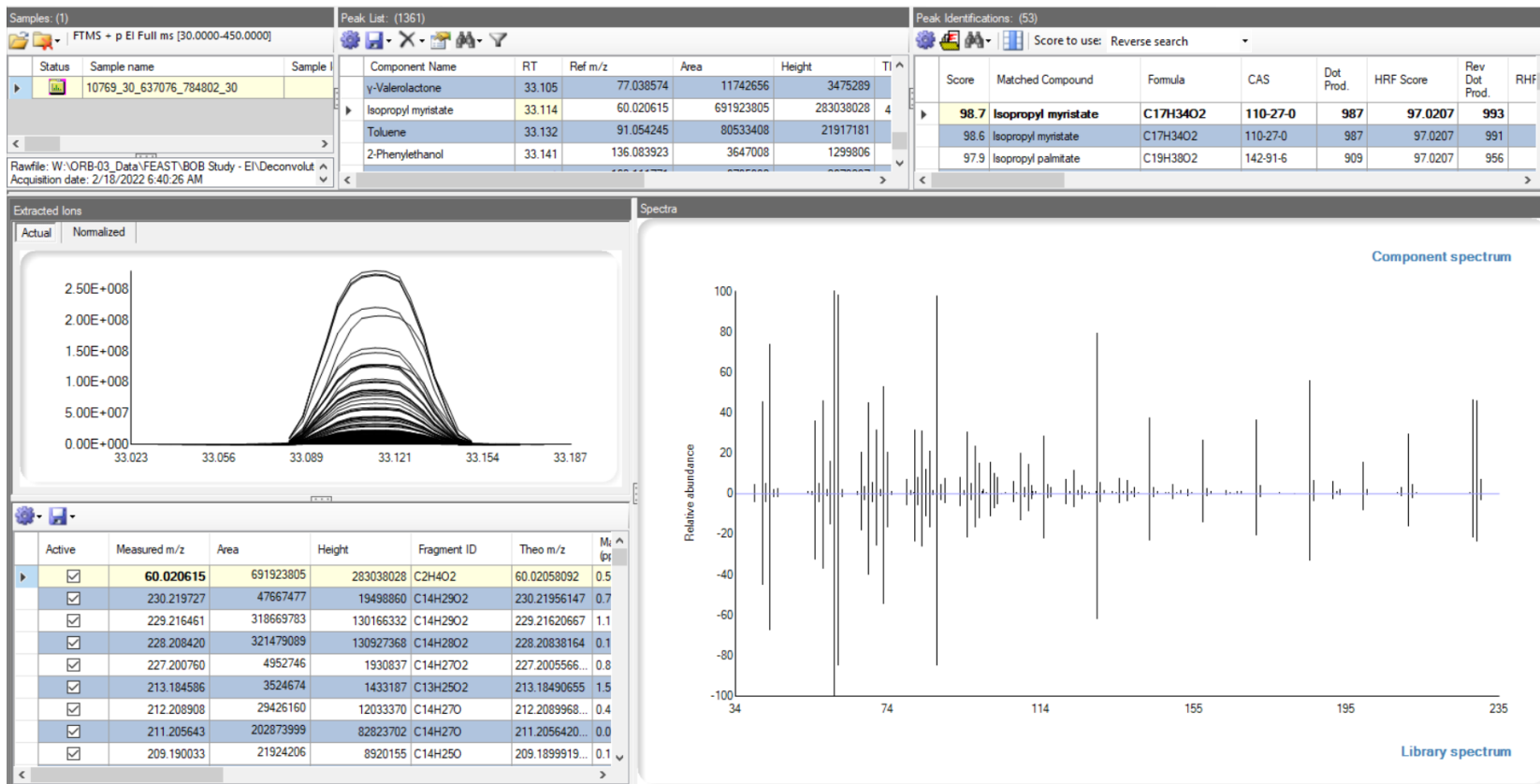


Deconvolved on-
breath feature @ RT
16.596

10937_30_798877_794607_30 (F156) RT=16.596, Deconvolved Spectrum, FTMS (+)



Example of a successful match (component vs in-house ATLAS library)



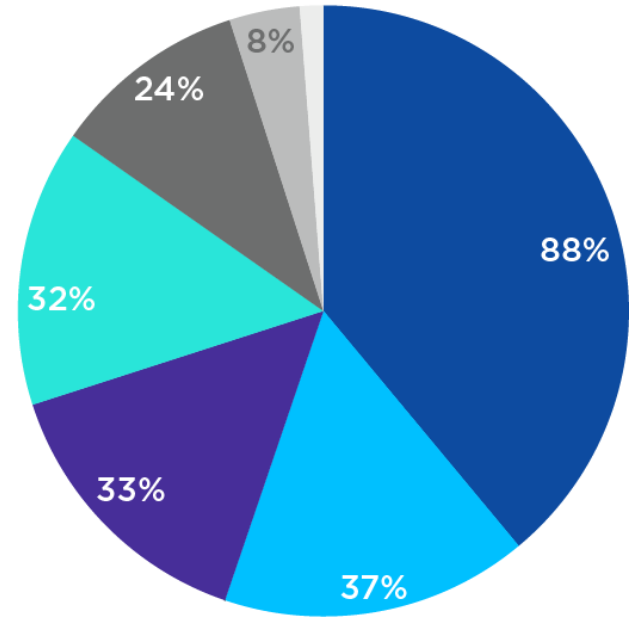
Summary stats: % of analysed standards successfully ID'd as on-breath

568 compounds analysed

432 compounds added to our in-house library

154 confirmed on-breath IDs!!!

- NIST matched to more than 1 feature and in HMDB
- NIST hit with SI score 800+
- NIST SI Score 800+ and in HMDB
- NIST hit with SI score 700+
- NIST top hit (regardless of score)
- Literature derived compounds
- NIST SI score between 550 and 700



Breadth of Atlas VOCs



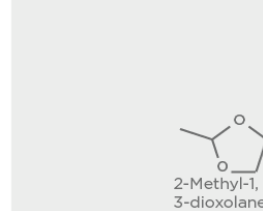
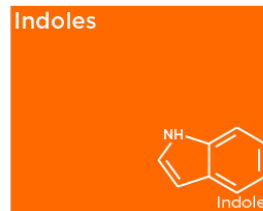
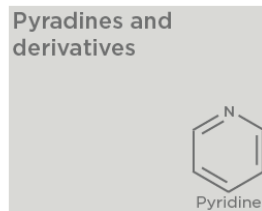
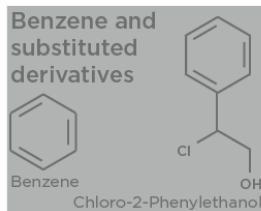
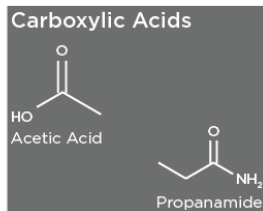
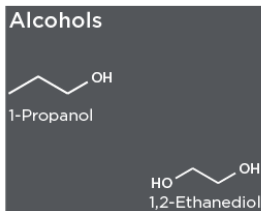
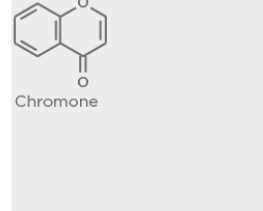
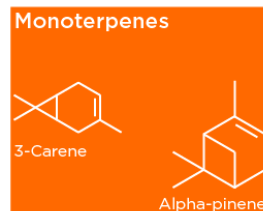
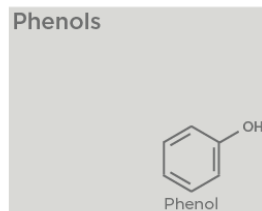
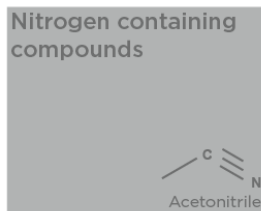
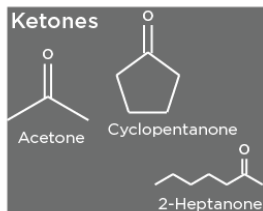
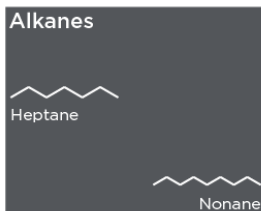
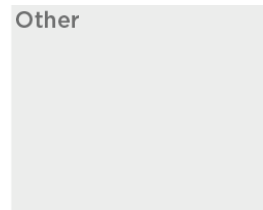
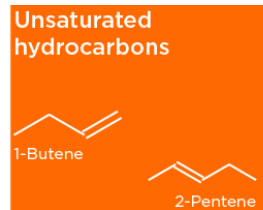
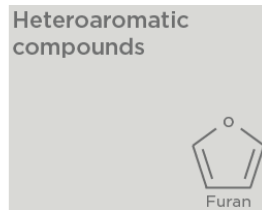
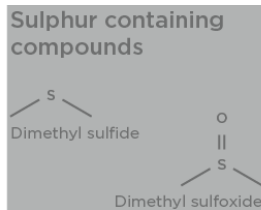
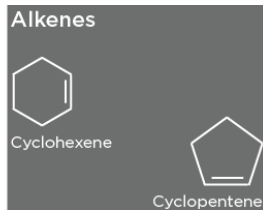
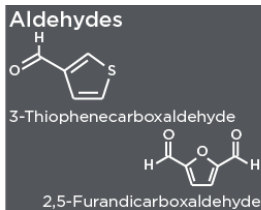
Retention time:
1.8 to 36.2 minutes



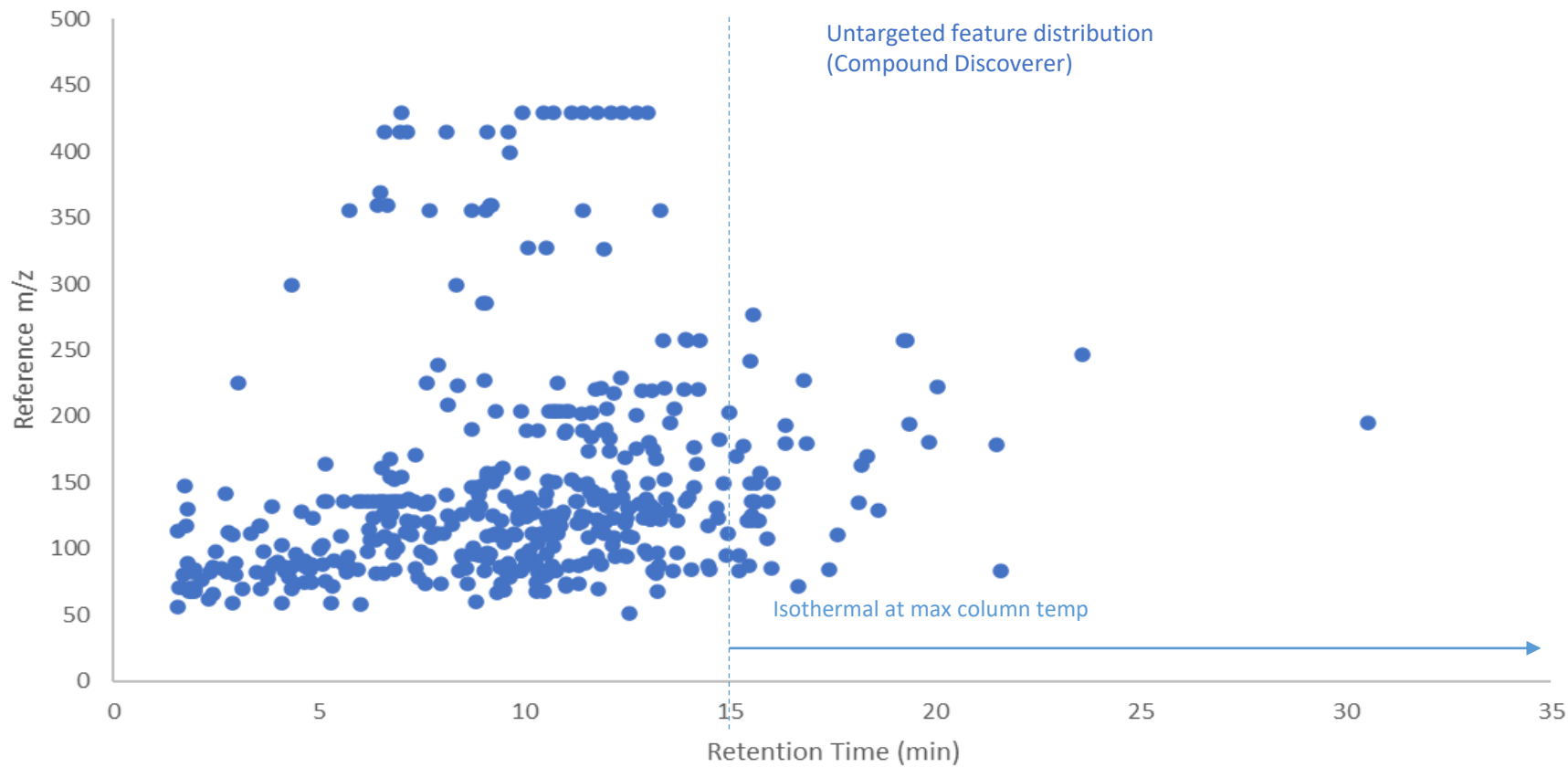
Molecular weight:
41 to 593



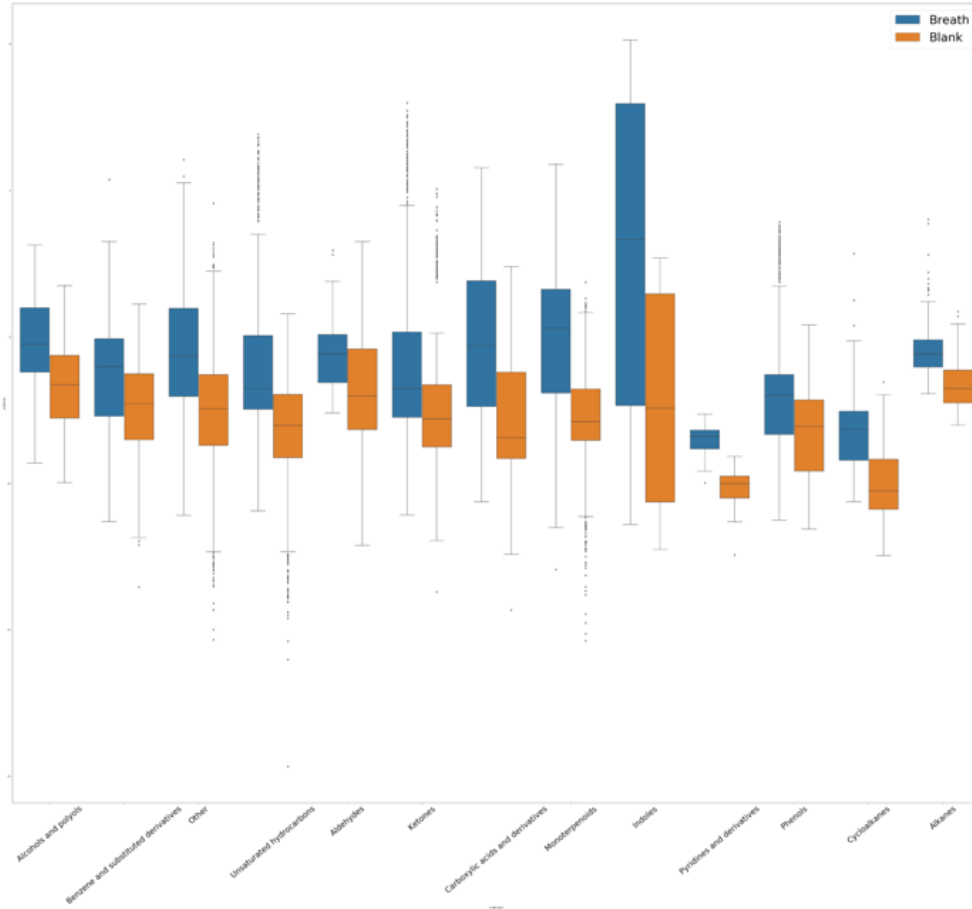
Polarity log kOW:
-1.4 to 8.2



Landscape of VOC's



Concentration range in breath



The Atlas today



Compounds Uploads Admin [Seen a bug/issue?](#) [General user feedback?](#)

[Login](#)

Compound Overview



Filter

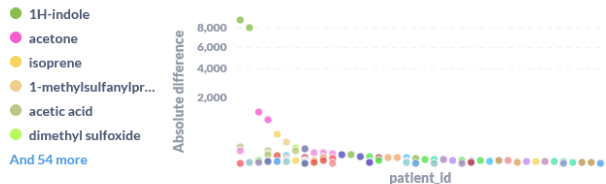
Reset

Owlstone name	Molecular formula	Literature	Num peaks	Min	Mean	Median	Max	Stats
Acetone	C ₃ H ₆ O	0	181	5.6	173.9	55.0	1567.5	
1H-Indole	C ₈ H ₇ N	0	181	0.8	1147.8	12.0	11269.3	
3-Phenylfuran-2,5-Dione	C ₁₀ H ₆ O ₃	0	181	0.5	5.3	4.7	16.7	
2-Methoxyethanol	C ₃ H ₈ O ₂	0	181	0.0	6.3	3.8	41.4	
Pyridine	C ₅ H ₅ N	0	181	0.7	7.7	3.4	53.8	
1H-Imidazole	C ₃ H ₄ N ₂	0	181	2.6	4.8	3.3	14.1	
Acetonitrile	C ₂ H ₃ N	0	181	0.1	4.5	3.0	66.6	
Acetic Acid	C ₂ H ₄ O ₂	0	181	0.0	18.5	2.8	203.7	
Methyl Acetate	C ₃ H ₆ O ₂	0	181	0.1	3.8	2.4	25.1	
Isoprene	C ₅ H ₈	0	181	0.3	74.6	2.0	581.4	
Propane-1,2-Diol	C ₃ H ₈ O ₂	0	181	0.1	3.1	1.9	17.9	
Formamide	CH ₃ NO	0	181	0.2	2.0	1.6	9.2	
Methyl Thiocyanate	C ₂ H ₃ NS	0	181	0.0	3.0	1.5	24.2	
2,3-Dihydrofuran	C ₄ H ₆ O	0	181	0.3	1.6	1.5	6.1	
Phenol	C ₆ H ₆ O	0	181	0.3	6.8	1.4	37.1	
Dodecamethylcyclohexasiloxane	C ₁₂ H ₃₆ O ₆ Si ₆	0	181	0.3	2.1	1.3	26.2	
2,6-Diisopropylbenz...	C ₁₂ H ₁₈ O	0	181	0.2	1.6	1.2	7.2	

The ATLAS today

Atlas Test Dashboards

Patients with unusual levels of compounds ?

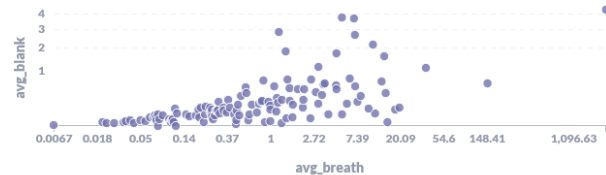


Blank vs Breath (with differences)

compound	avg_breath	avg_blank	diff
1H-indole	2,304.04	4.3	2,299.74
acetone	329.74	20.27	309.47
isoprene	149.47	0.62	148.85
acetic acid	36.04	1.11	34.92

Rows 1-4 of 127 ◀ ▶

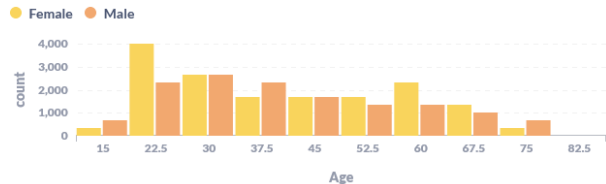
Breath vs Blank (scatter) ?



Distribution Breath vs Blank ?



Age, Sex Distribution ?



Conclusions

- The Breath Biopsy Atlas is a compendium of on-breath VOCs observed in a diverse cohort of UK adults
 - Analysed using Breath Biopsy Omni[®]
 - VOC IDs confirmed using chemical standards
 - 3 metrics distinguish on-breath VOCs from environmental background
- Atlas will serve as a reference dataset to facilitate biomarker discovery projects
- Atlas consists of +154 compounds confirmed to date, with multiple biological pathways represented
- Future work will expand the breadth of biological pathways and chemical classes included in the Atlas

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THANK YOU

