A new approach to Breath Biopsy® using TD-GC-Orbitrap

Breath Biopsy Conference

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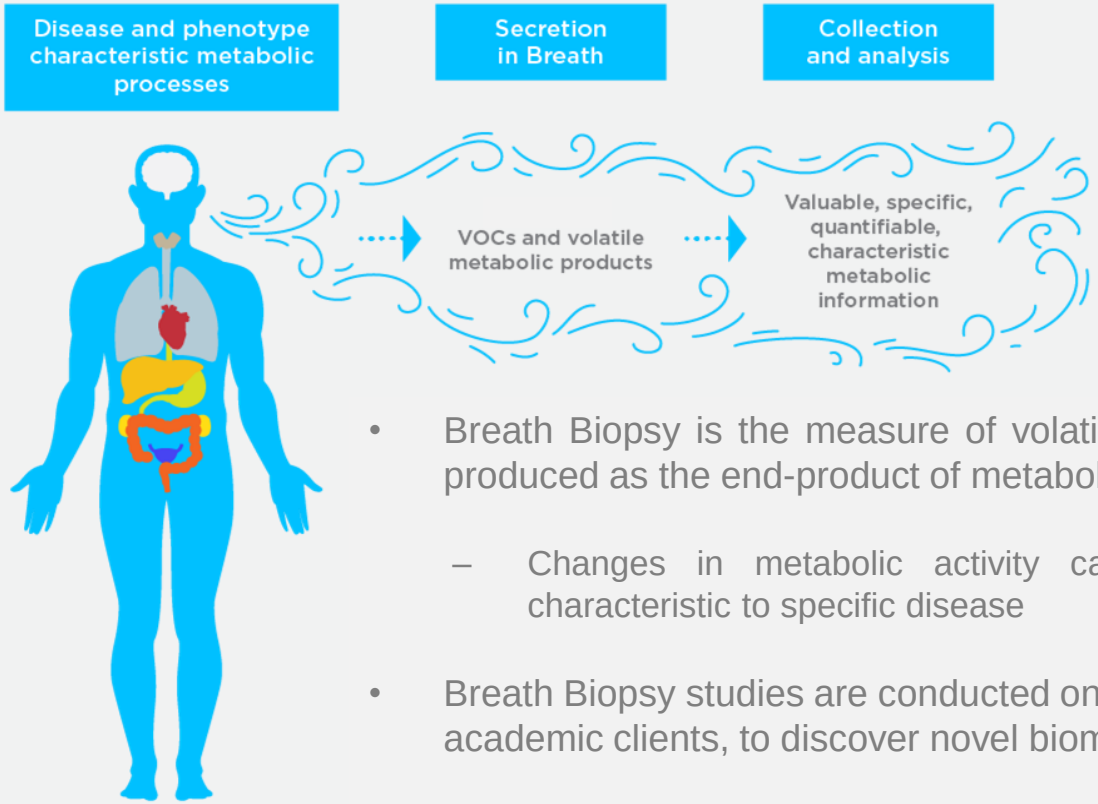
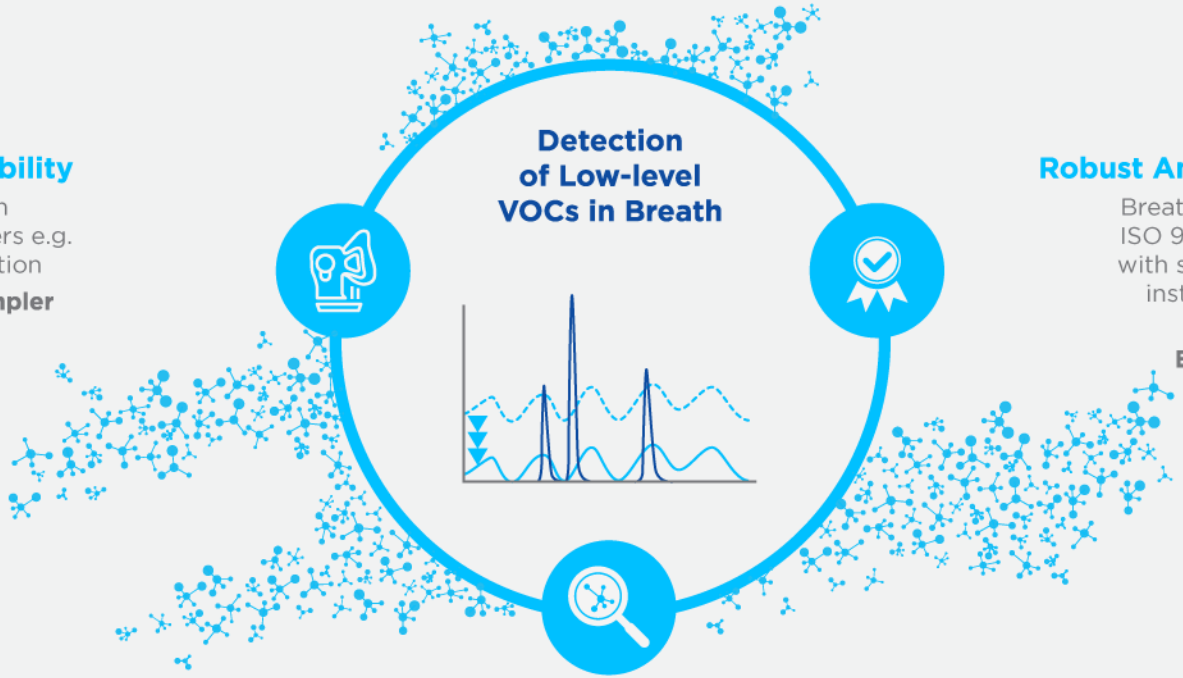
Breath Biopsy: measurement of VOCs characteristic of specific diseases



- Breath Biopsy is the measure of volatile organic compounds (VOCs), produced as the end-product of metabolic processes within the body
  - Changes in metabolic activity can produce patterns of VOCs characteristic to specific disease
- Breath Biopsy studies are conducted on behalf of pharmaceutical or academic clients, to discover novel biomarkers

What do we need to discover biomarkers from breath?



High Reproducibility

Standardized breath collection parameters e.g. breath volume, fraction

ReCIVA Breath Sampler

Robust Analytical Workflow

Breath Biopsy Laboratory in ISO 9001 certified company, with stringent QC checks on instruments, consumables and breath samples

Breath Biopsy Services

High Sensitivity

Rigorous conditioning process for consumables and filtered air supply ensure low background VOCs

Breath Biopsy Kits

CASPER Portable Air Supply

Breath Biopsy Services Workflow

CLINICAL SITE

Install **Breath Biopsy Collection Station** including **ReCIVA Breath Sampler** at clinical sites

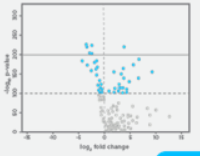
Regular supply of **Breath Biopsy Kits** including ready-to-use **Breath Biopsy Cartridge** and **Mask**

Collect **Breath Biopsy samples** and subject data in clinic

Store at 4 – 8 °C

Send **Breath Biopsy Cartridges** for analysis

Secure transfer of subject metadata to **Breath Biopsy digital biobank**



Comprehensive **discovery** analysis of candidate **VOC biomarkers**

Samples analyzed in **Owlstone Medical's Breath Biopsy Services Laboratory**

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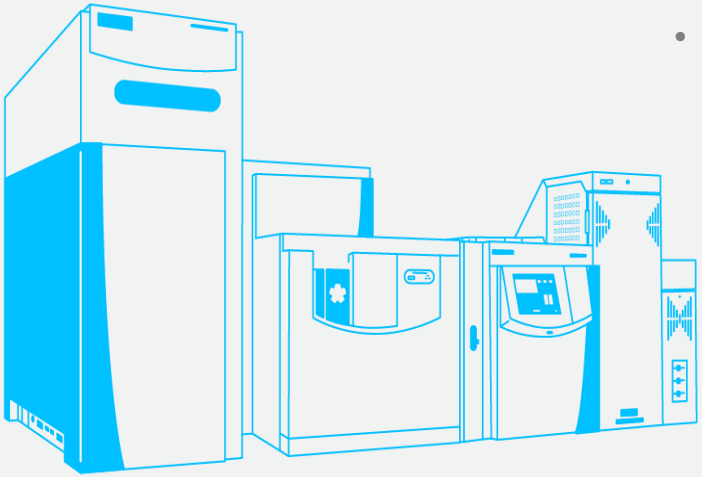
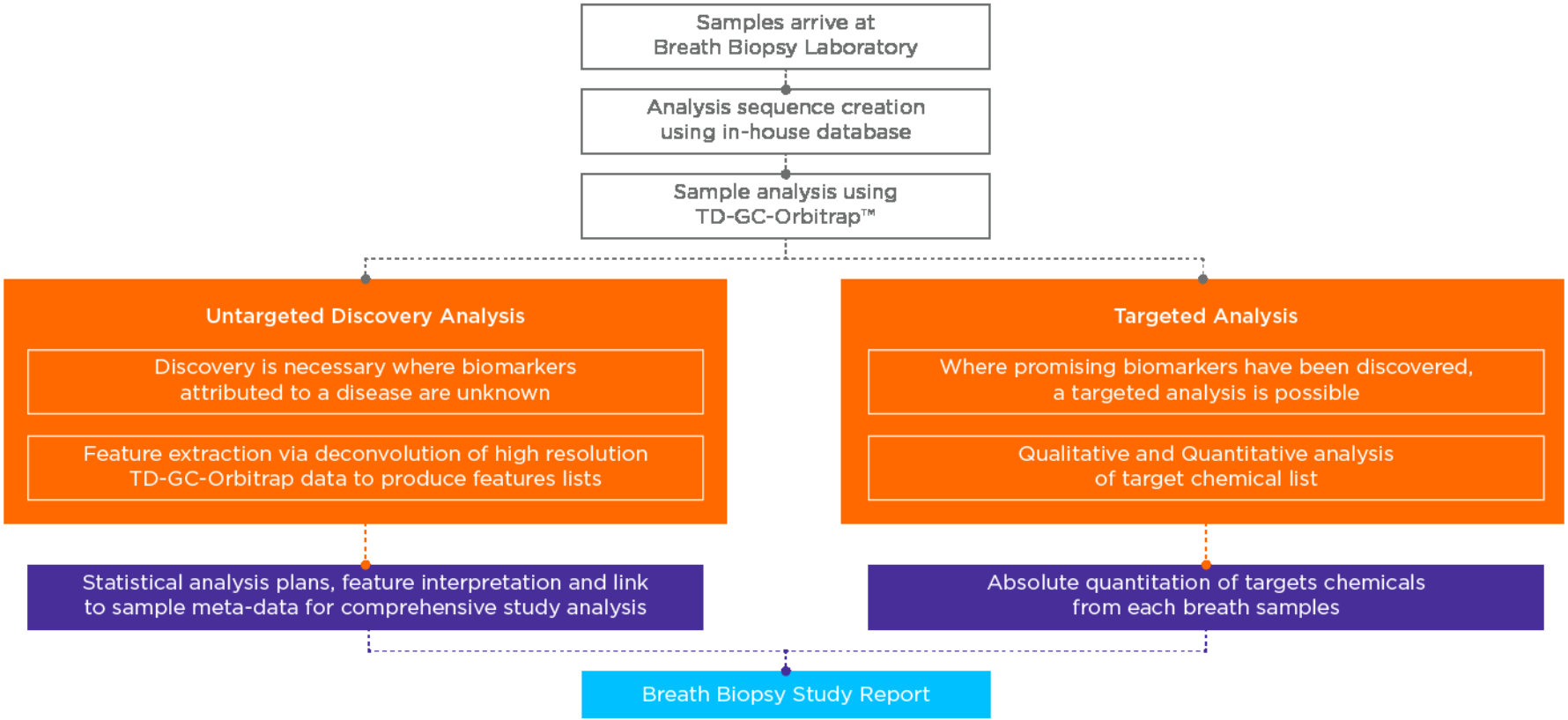
Integration of Orbitrap™ into Breath Biopsy platform

- TD-GC-MS is relied on as the “Gold Standard” analytical approach for breath analysis
  - GC provides separation of complex VOC mixtures
  - Capable of operating over wide analyte concentration range
  - High sensitivity allows detection of low abundance biomarkers
  - Mass spectra with accurate mass resolution provides structural information to facilitate identification of VOCs



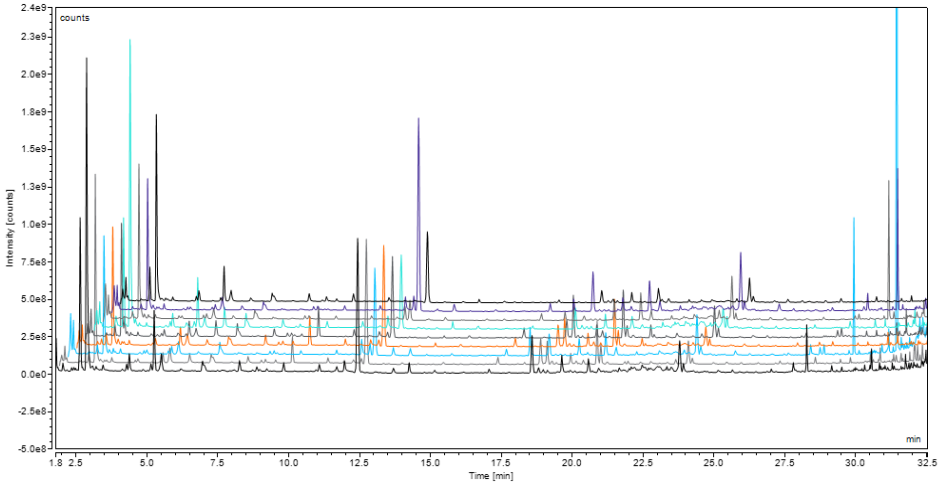
- Ongoing R&D collaboration with Thermo Scientific™ to integrate Q Exactive™ into Owlstone Medical’s Breath Biopsy platform
  - Qualify the mass analyzer and assess its ability to meet the needs of Breath Biopsy
  - Advance the application of non-invasive breath sampling, addressing widely accepted challenges associated with breath analysis

State-of-the-art breath analysis workflows

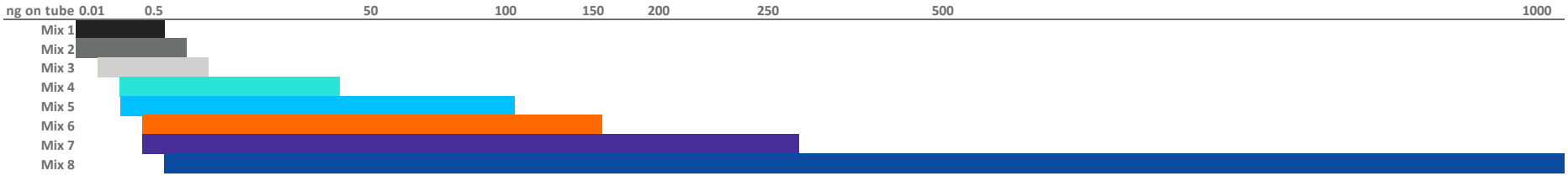


Breath Analysis challenge 1: Complexity of matrix

- Low-concentration analytes are to be detected in a complex breath matrix
- Requirement for the detection of both high-abundance VOCs and trace-level analytes within a single analysis



- Typical concentrations of chemicals in breath span 5 orders of magnitude



Analytical Methodology

Selectivity is determined by:

- The isolation width (quadrupole)
- The Orbitrap mass analyzer resolution
- The mass extraction window (mass accuracy)

Sensitivity is determined by:

- The number of ions measured
- The isolation width (quadrupole)

Scan speed is determined by:

- The Orbitrap mass analyzer resolution
- The maximum inject time/AGC target

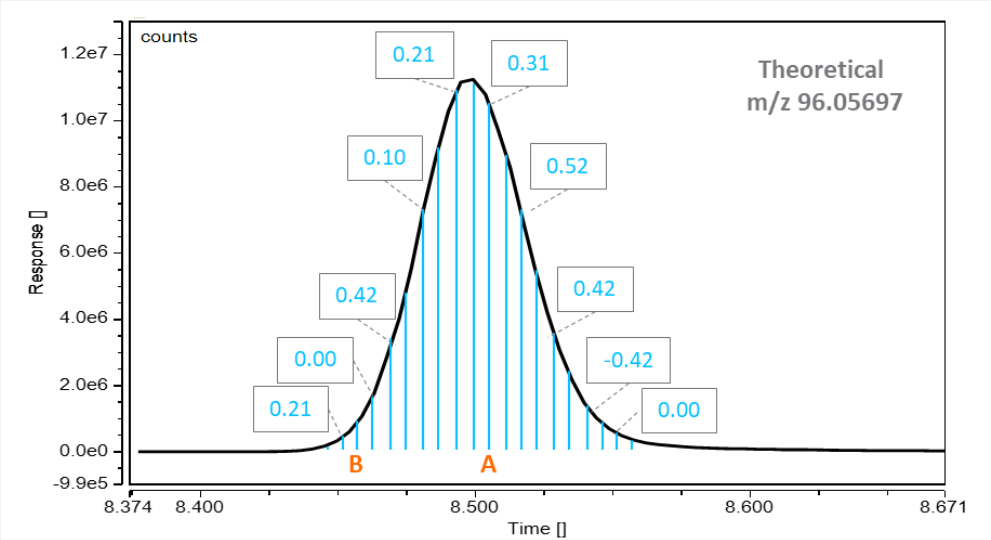
- Analytical methodology to enable both untargeted discovery and targeted analysis of breath requires a balance of selectivity, sensitivity and mass spectral scan speed

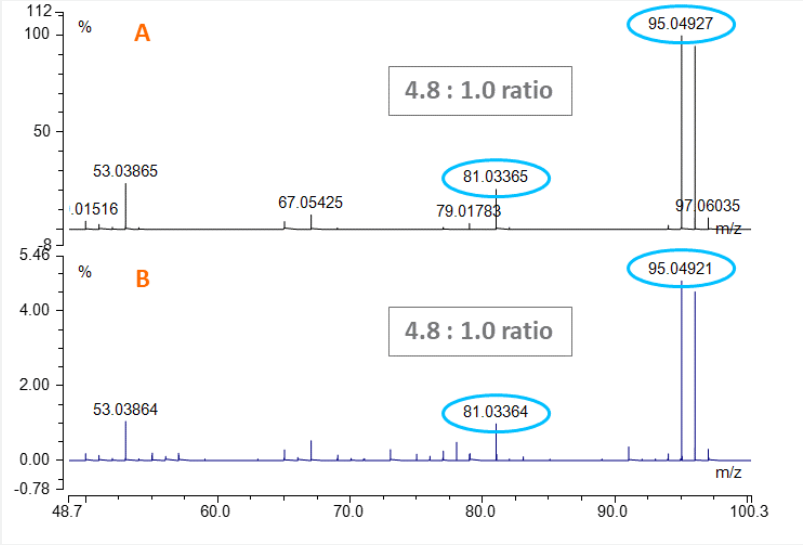
- Considerations for methodology for Breath Biopsy using TD-GC-Orbitrap:
  - Mass resolution
  - AGC target
  - Data scan rate
  - Scan mode (full scan / SIM)
  - Mass accuracy

- For example, utilisation of the C-Trap's AGC target can prevent overloading and space charge effects within the mass analyser due to high abundance VOCs

High Resolution Accurate Mass (HRAM)

- HRAM is crucial for untargeted metabolomics analyses for the detection of low-concentration analytes in complex matrix
- Sub-1 ppm mass accuracy can be achieved across the width of chromatographic peaks
- Ion ratio stability can be guaranteed at varying concentrations across the width of the chromatographic peaks

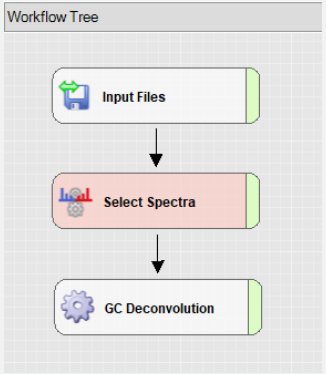


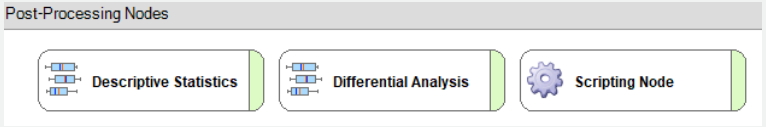


Breath Analysis challenge 2: untargeted discovery

- Untargeted discovery analysis is an important aspect of the breath biopsy workflow for metabolomics analysis and biomarker discovery
- The crucial challenge is reliable and reproducible deconvolution of fragmentation patterns of TD-GC-MS analysis in general

- Compound Discoverer v3.1:
  - Incorporates GC Deconvolution and statistical analysis into a single software
  - Includes option to embed custom scripts (python and R)
  - Ability to export flat feature table





Deconvolution output

- Features tables are important to Breath Biopsy to link output to study meta data
- Uniquely identify molecular features by:
  - Retention time
  - *m/z*
  - Response (peak area)

- Verification that the output of the deconvolution of the complex breath matrix is robust and reproducible is achieved using TD-GC-Orbitrap
  - Demonstrated via input of known chemical standards (below)

Name	RT [min]	Reference m/z	File name 1	File name 2	File name 3	File name 4
Feature 1			5.90E+08	5.79E+08	6.17E+08	5.88E+08
Feature 2			9.37E+08	8.94E+08	9.24E+08	1.82E+09
Feature 3			1.21E+08	1.20E+08	1.29E+08	1.20E+08
Feature 4			9.14E+08	8.83E+08	9.33E+08	8.78E+08
Feature 5			4.71E+08	4.60E+08	4.87E+08	4.56E+08
Feature 6			1.65E+08	1.58E+08	1.50E+08	1.31E+08
Feature 7			7.77E+08	7.38E+08	7.77E+08	8.05E+08
Feature 8			1.79E+08	1.75E+08	1.87E+08	1.71E+08
Feature 9			1.05E+07	9.67E+06	1.00E+07	9.02E+06
Feature 10			2.16E+07	1.84E+07	1.97E+07	2.08E+07
Feature 11			1.68E+08	1.64E+08	1.73E+08	1.74E+08
Feature 12			1.23E+08	1.20E+08	1.29E+08	1.29E+08

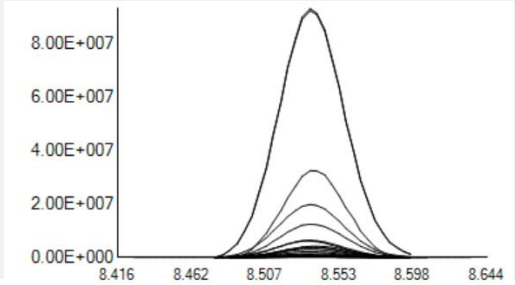
Response

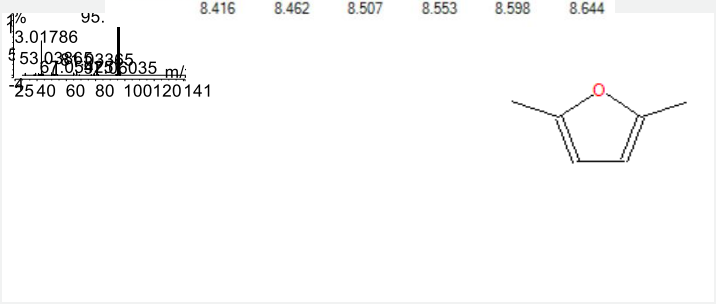
Data type	# of targets detected using targeted analysis	# of targets detected using deconvolution
Chemical standard	33/33 (100%)	32/33 (97%)
Spiked Breath sample	33/33 (100%)	32/33 (97%)

Breath Analysis challenge 3: Identification of unknowns

- Identification of unknowns against nominal mass NIST library is tentative
- High resolution filtering (HRF) and fragment prediction via accurate mass
- Sub-ppm mass accuracy can be utilised for biomarker identification through creation of accurate mass libraries
  - Allows comparison of compounds of interest across samples and between studies
  - Subsequent addition of compounds to library builds a wealth of high resolution reference spectrum for prospective and retrospective study analysis
- Combining NIST + accurate mass libraries can allow confidence in compound identification through Breath Biopsy analysis

Creation of accurate mass library





Analyse chemical standards on TD-GC-Orbitrap

Use **deconvolution** and predict fragment ions against theoretical masses utilising accurate mass

Add accurate mass spectrum to custom **Breath Biopsy Library**

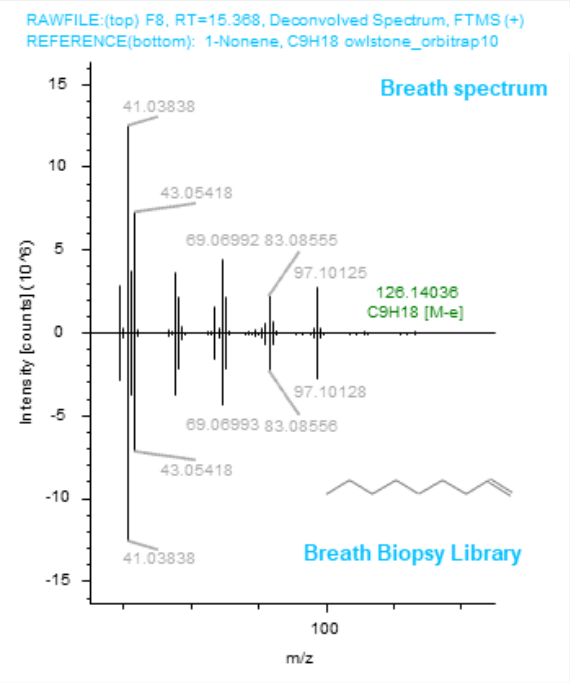
Comparison of breath spectra against the Breath Biopsy Library for **confident ID's**

Hide Related Tables

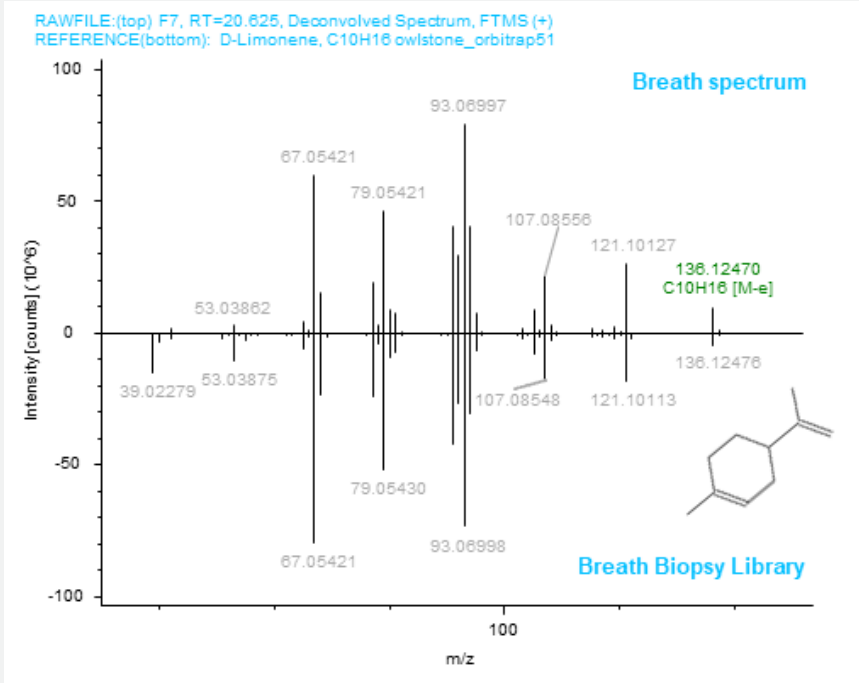
Annotated Fragments | Synonyms | Grouped deconvolved compound

	Checked	Source Library Hit	Empirical Mass	Formula	Mass Formula	Theo. Mass	Mass Error (PPM)	Is Mol Ion
3		owlstone_orbitrap18	96.056877	C6H8O	C(12)6 H8O	96.056966	0.926534	True
4		owlstone_orbitrap18	95.049171	C6H7O	C(12)6 H7O	95.049141	0.315626	False
5		owlstone_orbitrap18	95.044769	C6H6O	C(12)5 C(13)1 H6O	95.044671	1.033197	False
6		owlstone_orbitrap18	94.041359	C6H6O	C(12)6 H6O	94.041316	0.457246	False
7		owlstone_orbitrap18	82.036858	C5H5O	C(12)4 C(13)1 H5O	82.036846	0.148714	False
8		owlstone_orbitrap18	81.033569	C5H5O	C(12)5 H5O	81.033491	0.962564	False
9		owlstone_orbitrap18	80.025757	C5H4O	C(12)5 H4O	80.025666	1.137134	False

Untargeted use of accurate mass libraries



1-Nonene
Score 99.7
SI 993, RSI 995
HRF 99.3375, RHRF 99.3441



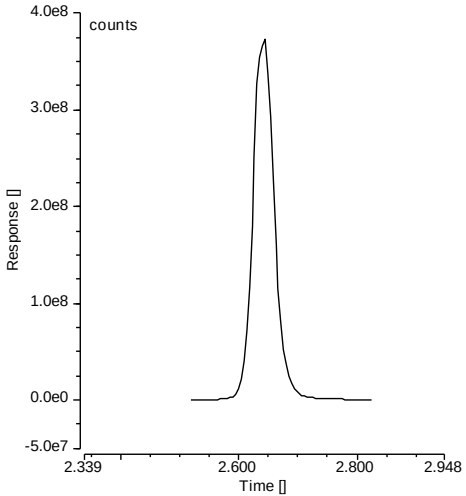
D-Limonene
Score 99.2
SI 972, RSI 976
HRF 99.1701, RHRF 99.1701

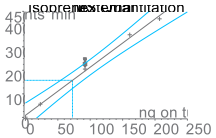
Targeted use of accurate mass libraries

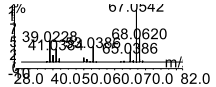
Peak detection using library reference spectra within RT window (targeted)

Quantitative calibration of target

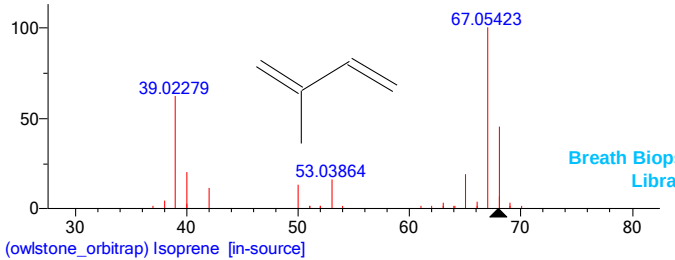
Confirmation of ID against library







Breath spectrum



Expanding content of Breath Biopsy accurate mass library

Pentane	2,5-Dimethylfuran	1-Decene	Hexamethyldisiloxane
Furan	2-Pentanone	Decane	Octamethyltrisiloxane
Isoprene	4-Methyl-2-pentanone	Octanal	Isopropyl alcohol
2-Methylpentane	1-Octene	Aniline	Benzene
Hexane	Octane	2-Ethylhexanol	3-Methylhexane
Butanal	Hexanal	Undecane	Methylcyclohexane
2-Butanone	Butyl acetate	n-Ethylaniline	o-Xylene
3,3-Dimethylpentane	2,3-Dimethylheptane	Dodecane	D-Limonene
2-Methylbutanal	1-Nonene	p-Menthone	
Heptane	Nonane	Decanol	
Trichloroethylene	4-Methylnonane	Dodecanol	

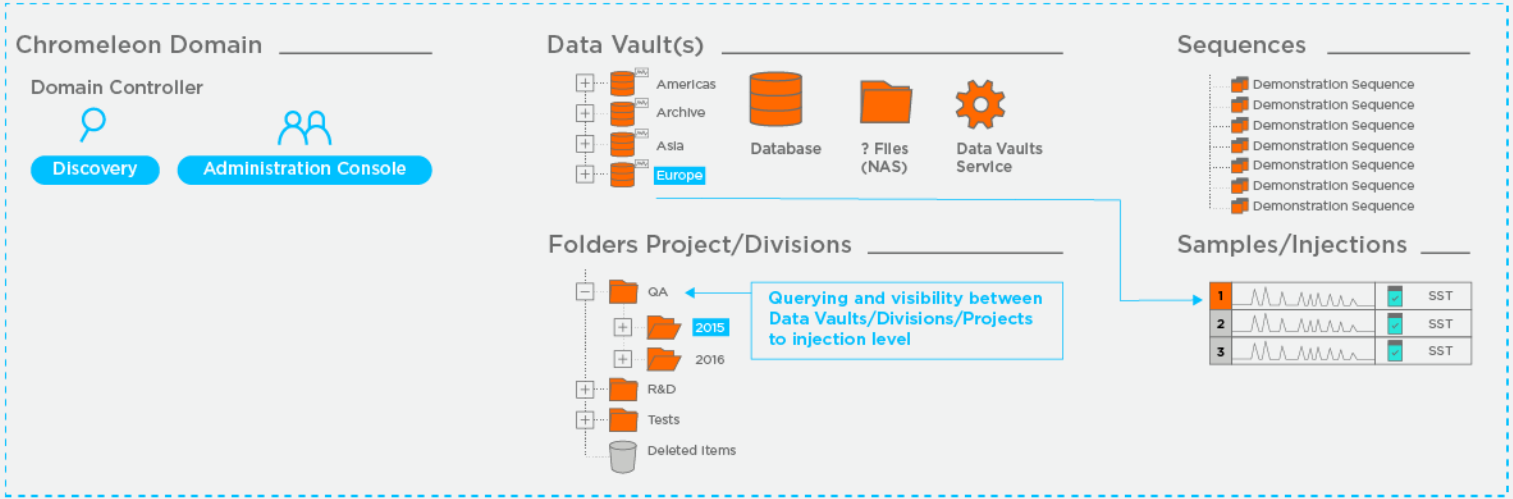
Breath Analysis Challenge 4: high-throughput service analysis

- High throughput service lab
  - Instrument uptime and real-time status
  - Quality process implementation and maintenance
  - Data management

- **Hardware**
  - Removable ion source – only interchangeable part
  - Dual filament
  - Means reduced downtime, minimal user interaction

- **Software**
  - Chromeleon Chromatography Data System (CDS)

Chromeleon CDS Enterprise and data management



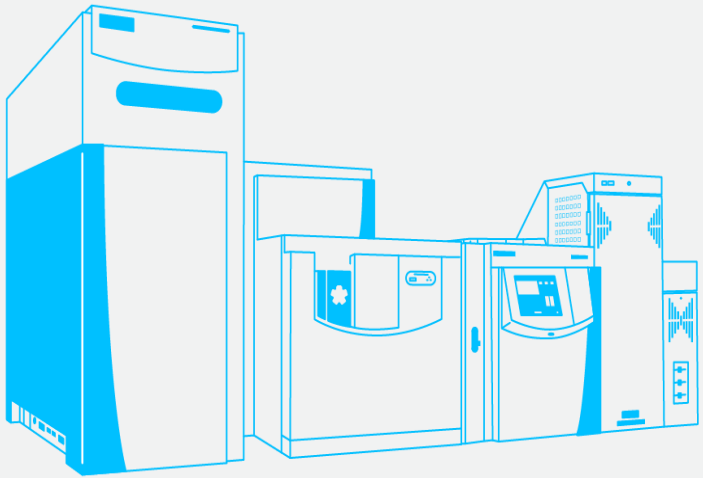
- Chromatography data system (CDS) implementation into Breath Biopsy services workflow for:
  - Management of analytical processes
  - Instrument control
  - Raw data storage (cloud)
  - Result reporting

- Enterprise network allows:
  - Linking of instruments across laboratories
  - Control of user access
  - Data integrity
  - Compliance:
    - GLP, GMP and 21 CFR Part 11 regulations

Chromeleon: Unified workflow via single software

Instrument control

- Online data acquisition and processing
- System suitability test and intelligent run control

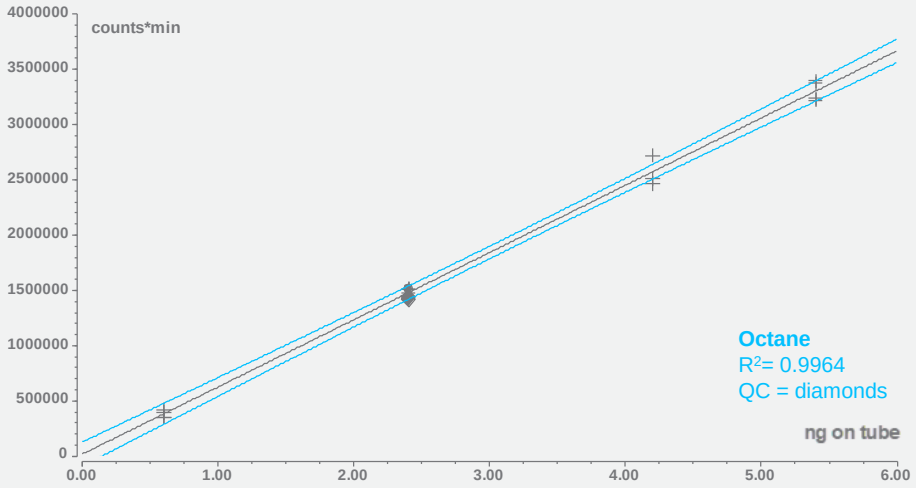


Chromeleon: Unified workflow via single software

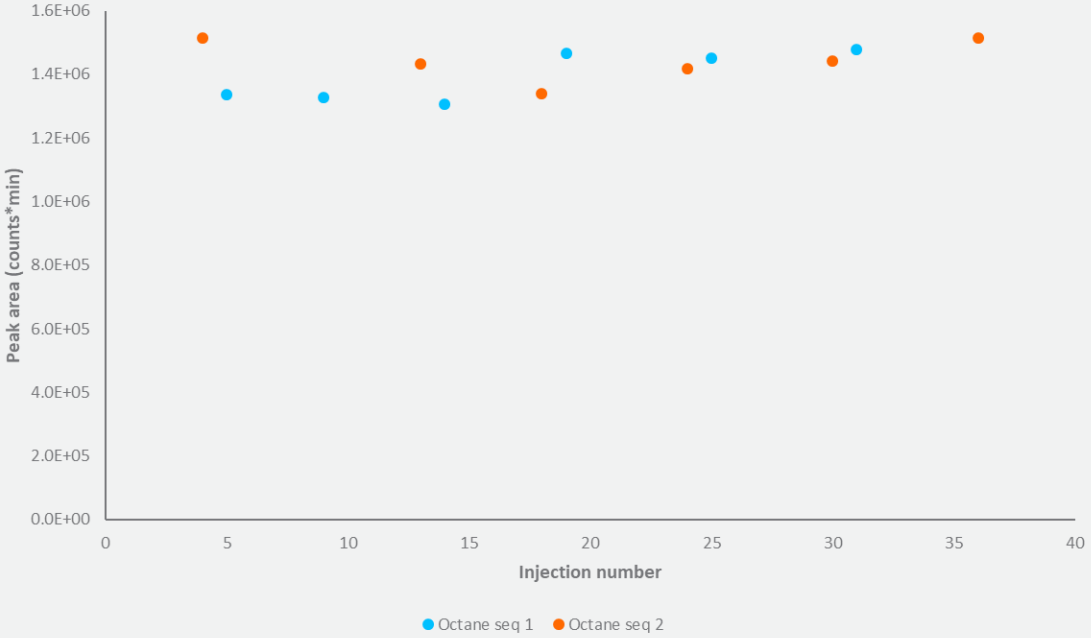
Instrument control	• Online data acquisition and processing • System suitability test and intelligent run control
Targeted data processing	• Qualitative and quantitative calibration • Data review with customisable view settings

Targeted data processing

- Perform automated qualitative and quantitative data processing
- QC throughout the sequence
- Automated monitoring of system suitability tests to ensure data quality



Importance and examples of QCs



- Good intra- (<4.8%) and inter- (5.2%) sequence precision ensures analytical variability is low
- Average accuracy of QC is 98.4%, giving confidence in standard preparation

Chromeleon: Unified workflow via single software

Instrument control	• Online data acquisition and processing • System suitability test and intelligent run control
Targeted data processing	• Qualitative and quantitative calibration • Data review with customisable view settings
Audit trail	• Instrumental log and sequence monitoring • User accountability

Example audit trail sequence report

Instrument:QE-GC Sequence: XXX.seq

Audit Trail

Injection Details

Injection Name:	**YYYYMMDD_ORB-02_XXXX_Demonstration**	*Run Time (min):*	**38.50**
Vial Number:	**100**	*Injection Volume:*	**1.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	***XXX***		
Processing Method:	***XXX***		
Injection Date/Time:	**23/Sep/19 18:22**		

BREATH® BIOPSY

Day Time (UTC+01:0	Ret. Time min	Command/Message
18:01:31		Audit trail of injection "chrom://fpzpnr2/ChromeleonLocal/Instrument Data/QE-GC/ XXX.seq
18:01:31		Start of injection 1 " **YYYYMMDD_ORB-02_XXXX_Demonstration** ", using instrument method
18:01:35	0.000	Entered stage "Instrument Setup"
18:01:35	0.000	Trace_1310.Back_SSL.FlowMode = FlowCtrl
18:01:35	0.000	TD.MethodMode = 23StageDesorb

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Instrument control	• Online data acquisition and processing • System suitability test and intelligent run control
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Example of user roles and permissions

BBL Analyst

- Instrument operation
- Sample sequence set-up
- Data acquisition in accordance with quality procedures
- Initial data review and report creation
- Submit data to data quality manager for further review

SUBMIT

Data Reviewer

- Qualitative and quantitative data review of target analysis
- System suitability test result review
- Reporting data quality to:
  - BB database
  - Study project managers
  - BBL manager

REVIEW

BBL Manager

- Final approval of data reporting
- Assignment of roles within the BBL
- Ensuring BBL staff compliance with quality standards
- Certifying instrument performance and laboratory uptime

APPROVE

Chromeleon: Unified workflow via single software

Instrument control	• Online data acquisition and processing • System suitability test and intelligent run control
Targeted data processing	• Qualitative and quantitative calibration • Data review with customisable view settings
Audit trail	• Instrumental log and sequence monitoring • User accountability
Data reporting	• Breath Biopsy customisable reports • Automated generation of reports

Summary

- Described how adopting Orbitrap™ mass analyser and associated software packages has facilitated significant advancements in Breath Biopsy®

GC-Q Exactive™	•The Orbitrap™ is able to detect high-abundance VOCs and trace-level analytes due to high dynamic range and flexible scan modes •High resolution accurate mass means ion ratio stability across chromatographic peaks
Compound Discoverer 3.1	•Provides ability to export flat features tables that can be linked to study meta data •Allows reliable and reproducible deconvolution of TD-GC-MS fragmentation patterns •Deconvoluted accurate mass spectra can then be used build libraries for confident IDs
Chromeleon (CDS)	•Management of analytical processes •Instrument control •Raw data storage (cloud) •Result reporting • Linking of instruments across laboratories • Control of user access • Data integrity

Summary

- Work described is part of ongoing R&D collaboration with Thermo Scientific™ to further optimise at Owlstone Medical's Cambridge, UK, Breath Biopsy laboratory
- Sample analysis using Orbitrap™ mass analyser begins in January 2020 for selected Breath Biopsy studies, with wider roll out across 1st half of 2020
- For more information on GC-Q Exactive™
  - **Cristian Cojocariu**
- For more information on Breath Biopsy®
  - **Huw Davies**

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THANK YOU

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