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An Integrated Platform Technology for Volatile Biomarker Discovery

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Introduction

Changes to the pattern of individual's volatile metabolites (volatilome) are observed in, and may be used to understand the biochemical processes associated with, metabolic disorders (including those resulting from nutrition, lifestyle choices) and disease (cancer, immune & inflammatory, infection & pathogenesis).

Complex changes to the volatilome result from exogenous and endogenous stimuli including physiological state, environmental, genetic, infection and disease.

Volatile compounds are present in breath, bio-fluids & secretions, stools and tissue samples and consistent, clinically diagnostic profile changes may be detected at the earliest stages of disease. These changes have the potential to facilitate minimally invasive (breath, saliva, urine, stools) sampling for diagnosis and early treatment, improving prognosis and patient outcomes and reducing healthcare costs.

Whilst there have been many research studies demonstrating that volatile compounds are biomarkers of disease, the FDA has only approved three diagnostic breath tests in the last 20 years: breath hydrocarbons to monitor for heart transplant rejection, the Urea breath test for H.pylori infection and the NO test for asthma therapy.

One of the main reasons that studies have failed to translate from promising basic and applied research to clinical use has been the reliance on 40-year-old thermal desorption technology: this cannot deliver the data quality, sample throughput or analytical capacity required to demonstrate the clinical specificity, sensitivity and selectivity required to do the validated studies needed to obtain regulatory approval.

In this poster, we present Anatune's innovative, integrated platform technology for comprehensive volatile biomarker discovery.

Sample Stability

Bioactive volatile metabolites include carbonyls, volatile fatty acids, sulphur and nitrogen compounds. Many of these are labile, reactive or liable to adsorb on active surfaces in the instrumental sample flow-path.

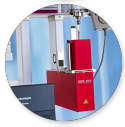
To maximise sample stability, Anatune Volatilomics solutions can be configured with cooled stacks or sample trays for the storage of both sample vials and TD tubes.

Our Volatilomics Solutions feature Agilent's Inert Flow Path (to minimise adsorptive losses) and GERSTEL Thermal Desorption (TD) Technology.

Both the GERSTEL TDU2 and TD3.5+ benefit from forward flushed secondary trapping and heating of the entire length of the TD tube, maximising analyte recovery and transfer to the GC column. The TDU design provides the best available solution for moisture management.

Enrichment Capabilities

Whilst a few metabolites can be determined by static headspace, most volatile metabolites are typically present at low levels (e.g., low to sub-ppm(v) concentrations in breath). For GC-MS analysis enrichment is normally necessary.



All techniques, widely used in volatilomics studies are integrated into to the Anatune Volatilomics Solution:

- Static Headspace - plus hot injection and trapping (HIT)
- Solid Phase Microextraction (SPME)
- Headspace Sorptive Extraction (GERSTEL Twister®)
- Thermal Desorption (3.5 x ¼" tubes)
- True Dynamic Headspace (DHS) plus
  - Full-evaporative technique (FET)
  - Multi-Volatile method (MVM): the gold-standard for volatile biomarker discovery

Solutions can be configured for automated internal and recovery standard spiking of both liquid samples and TD tubes. The preparation of calibration curves can be fully automated.

Detection

Combining High-Resolution Accurate Mass GC/Q-TOF and olfactometry to Identify and characterise Parkinson's disease Biomarkers

The Anatune Metabolomics Solution is available with all current Agilent GC-MS Platforms:

7890/5977 GC-MSD
- Discovery and targeted Volatilomics studies

7890/7000 GC-QQQ
- Targeted and semi-targeted studies (e.g, biomarker verification/ validation)

7890/7250 GC/Q-TOF
- Full-scan High-Resolution Accurate-Mass GC/Q-TOF with low energy EI capability
- Includes molecular formula generator and molecular structure correlator for biomarker ID

Human olfactory detection (ODP) and Insect electroantennography (EAG) options are available for bio-active volatile identification.

Data Analysis & Reporting

Anatune Solutions for Volatilomics include fully automated, just-in-time, sample preparation to ensure that the rate limiting step to sample throughput is the instrumental runtime.

They also feature integrated feature extraction, data analysis, reporting and multivariate statistical analysis for full, end-to-end workflow automation:

- Agilent MassHunter software for routine targeted analysis
- Agilent Profinder® for automated feature extraction
- Specialist libraries of volatile metabolites (optional)
- Agilent Mass Profiler Professional (multivariate statistics) package
- In-built Pathway Architect to map features to pathways
- Integrated multi-omics data modelling in Agilent GeneSpring

Combining fully automated sample preparation, design of experiments (DoE) and multivariate statistical analysis provides the Golden Triangle for volatilomics studies.

This Golden Triangle allows robust, repeatable and reproducible methods to be rapidly developed, optimized and validated in the R&D laboratory to ensure that methods are ready for scale-up to clinical research studies.

Conclusions

The Anatune Volatilomics Solution is a flexible, modular, user-programmable platform for Volatile Biomarker Discovery, verification and validation studies featuring:

- Controlled temperature sample storage, inert sample path, forward flushed secondary trapping and enhanced tube heating for optimal performance
- Comprehensive options for selective enrichment for targeted metabolite studies - static headspace, HIT, SPME, Headspace Twister®
- Thermal Desorption and Dynamic Headspace (Multi-Volatile Method) for comprehensive untargeted discovery volatilomics
- Automated preparation of calibration standards, plus internal standard and recovery standard spiking
- Choice of GC-MSD, GC-QQQ and GC-Q/TOF platforms
- Highly configurable - multi-method low throughput solutions for core facilities to high throughput single method solutions for large scale studies

Whilst this poster is focused on Clinical Research Studies, the Anatune Volatilomics solution can be used for volatilomics studies in Synthetic Biology, Industrial Biotechnology, monitoring of agricultural and biogenic emissions, chemical ecology studies and microbiology (microbiome).