anatune
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The Anatune Metabolomics Prep Station

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Introduction

Figure 1 – Typical online (LC-QQQ) Metabolomics Prepstation
Modules include evaporation station and solid phase extraction

Robust and reproducible sample preparation methods are fundamental to the delivery of high quality metabolomics and metabolic phenotyping datasets.
Whilst the last decade has seen great improvements in instrumental precision, sensitivity and robustness; analytical throughput is limited by the twin bottlenecks of sample preparation and data processing: both are labour intensive and time-consuming.
Whilst some laboratories have automated sample preparation to reduce cost and human error, most sample preparation is still done manually, and is a significant source of experimental error. Where automation has been adopted, systems are either dedicated liquid handling systems or expensive, bespoke systems designed to automate a single protocol in batches.
Both options have significant limitations:

- Liquid Handling systems lack the capabilities (e.g., evaporator, centrifuge) to fully automate routine sample preparation tasks
- Bespoke systems lack the flexibility to deal with different sample/matrices as laboratory, or regulatory needs, change
- Preparing samples in batches results in exclusion of unstable metabolites (or their derivatives) from the results

To eliminate these limitations, we have developed a flexible, modular metabolomics PrepStation that can configured to automate the full range of NMR, LC/LC-MS & GC/GC-MS sample preparation methods including the Fiehn Protocol for biphasic extraction and derivatization (MOX-TMS and tBDMS) and the Folch/Bligh & Dyer extraction, lipid class fractionation and derivatization.

Sample Extraction

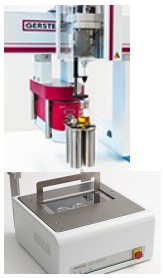

Figure 2 – GERSTEL multi-position vortexer (mVORX) and Robotic Centrifuge (CF-200)

[1,2]Metabolite extraction protocols (common to NMR, LC-MS and GC-MS platforms) are well established. They involve either simple or biphasic tissue extraction, with thorough mixing for reproducible and repeatable extraction, and centrifugation to sediment solids and/or disperse the emulsion formed and clarify the phase interface.
We have [3]recently demonstrated that the GERSTEL QuickMix™ and Anatune Robotic Centrifuge (CF-200) can used to fully automate both metabolite extraction and tissue homogenisation (using commercially available tissue lysis matrices). Where more tissue (≥50mg F.W) is required to obtain a representative sample, samples may be pre-homogenised off-line and loaded onto cooled sample trays on the GERSTEL MPS.

Evaporation & Derivatization

Whilst polar extracts for NMR or LC-MS often require no further sample preparation, heat-shock step may be used to denature enzymes.
Non-polar NMR and LC fractions, together with both polar and non-polar fractions for GC/MS are typically evaporated to dryness. For LC and NMR extracts are reconstituted in LC mobile phase; for GC-MS the dried extracts are derivatized, typically using two-step (MOX-TMS) derivatization. Heat-shock, evaporation/oximation (MOX) and silylation (TMS) steps can be automated in using the GERSTEL MultiPosition Evaporation Station (mVAP) and Incubator/Agitator respectively.
Adding the GERSTEL Ultrasonic option allows ultrasonically assisted derivatization (e.g., tBDMS)

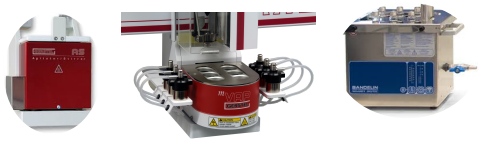

Figure 3 – GERSTEL Agitator, MultiPosition Evaporation station (mVAP), and Ultrasonic Option

Just-in-Time With PrepAhead

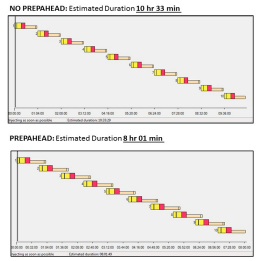

Figure 4 – Maestro PrepAhead for just-in-time sample preparation

The Anatune Metabolomics PrepStation features full instrument control by GERSTEL Maestro® Software. The system can be configured as a standalone PrepStation, or installed online with Agilent Technologies® LC/LC-MS and GC/GC-MS systems to deliver integrated metabolomics platforms. Maestro uses Prep-Ahead® (Figure 4) for full overlap of sample preparation, offset by the LC/GC cycle time.
With just-in-time sample preparation, the system delivers a freshly prepared sample to the instrument at the end of the previous GC/LC run: minimising, or even eliminating, sample/derivative stability effects.

Data Analysis & Reporting

Anatune Solutions for metabolomics and metabolic phenotyping 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 to eliminate the second (data analysis and reporting) bottleneck built on:

- Agilent MassHunter
- Agilent Mass Profiler Professional
  - Cross platform (GC-MS, LC-MS/MS, SFC-MS/MS) solution
  - Integration with METLIN®, an accurate mass LC/Q-TOF library
  - Full integration with SimLipid® for LC-QQQ lipidomics
- Sherlock® X from MIDI Inc. for turnkey solutions for LC, GC and GC-MS:
  - Microbial ID for routine microbial metabolic phenotyping
  - [4]Soil Plasma Phospholipid Fatty Acid (PLFA) analyses for soil microbial community analysis
  - Flexible FAME analysis solution with custom reporting for:
    - Nutrigenomics studies, Aquaculture and crop breeding
    - Food testing nutritional composition analysis
    - Metabolic Engineering and Synthetic Biology
- In-built Pathway Architect to map features to pathways
- Integrated multi-omics data modelling in Agilent GeneSpring

Conclusions

The Anatune Metabolomics PrepStation is a flexible, modular, user-programmable platform for Metabolic Phenotyping and Metabolomics featuring:

- Fully-automated, just-in-time, sample extraction and preparation for virtually any metabolomics/metabolic phenotyping protocol (off-the shelf and bespoke solutions)
- Offline sample preparation (NMR, LC/LC-MS, GC/GC-MS) or online sample preparation with all Agilent platforms (GC, HPLC, uHPLC, SFC; MSD, QQQ, Q-TOF, IM/Q-TOF)
- Combining GERSTEL, Agilent and MIDI Solutions delivers true end-to-end automation for Metabolomics and Metabolic Phenotyping
- Highly configurable - multi-method low throughput solutions for core facilities to high throughput single method solutions for large scale studies
- Automation delivers improved data quality, faster results, increased grant income, reduced overheads and increased productivity (e.g., more, higher impact publications).

References:

1. Folch J. et al, J. Biol. Chem. (1957), 226: 497-509
2. Bligh E.G., & Dyer W.J., Canadian Journal of Biochemistry and Physiology (1959), 37: 911-917.
3. Liscio, C., Fully automated on-line Folch Extraction and trans-methylation of fatty acids in salmon tissue. Anatune Chromatography Technical Note, AS157 (2015)
4. Buyer J.S. & Sasser M., (2012) High Throughput Phospholipid Analysis of Soils, Applied Soil Ecology 61: 127-130

Anatune solutions are available from Anatune in the UK and Ireland, and worldwide through GERSTEL, its subsidiaries and distributors