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PrepAhead: how to master your time with GERSTEL Maestro software

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Introduction

In many analytical workflows, sample preparation is often the most time-consuming step. Also, workflows using techniques such as Dynamic Headspace (DHS) or Solid Phase Micro Extraction (SPME), where the sample preparation is automated by default within the GC-MS analysis and usually not particularly lengthy (incubation times are usually contained within 20 minutes), can be significantly affected timewise in a high throughput context.

As a rule of thumb, good time management is driven by the capability of multitask that is by definition the ability to perform several tasks at a time. And that is exactly what the PrepAhead function within MAESTRO software does. Using the MAESTRO software PrepAhead function, sample preparation steps are performed during analysis of the preceding sample for best possible system utilization and highest sample throughput. This approach not only eliminates downtime but it also guarantees samples to be freshly prepared just before analysis limiting biases introduced by potential degradation over time of the target analytes (e.g. TMS derivatives in GC-MS analysis or photosensitive compounds). Figure 1 shows schematics of how PrepAhead works.

Figure 1: Comparison between standard approach and PrepAhead function available within GERSTEL Maestro Software

This application note showcases some examples of the use of Maestro PrepAhead function for the automation of relevant analytical workflows.

Instrumentation

Example 1: DHS-GC-MS for the analysis of Parkinson's disease skin swab samples

- *Autosampler:* GERSTEL MPS xt Dual Rail
- *Modules:* Vial tray VT32-20mL, GERSTEL DHS module
- *GC-MS:* Agilent GC 7890- MSD 5977BC, HES Source

Example 2: Online MOX-TMS derivatisation of biological samples for untargeted metabolomics

- *Autosampler:* GERSTEL MPS xt Dual Rail,
- *Modules:* Agitator, GERSTEL MultiPosition Vortexer (mVorx), GERSTEL MultiPosition Evaporation Station (mVAP), Anatune CF-200 Robotic Centrifuge
- *GC-MS:* Agilent GC 7890-5975C MSD, Inert Source EI

Example 3: Online sample preparation for the analysis of tablets by LC-UV

- *Autosampler:* GERSTEL Dual Head MPS system
- *Modules:* MPS xt Stainless Steel Injection Valves Cheminert - 2x 3 Solvent reservoirs 100 mL (total of 6 positions) - Modular wash station - Tray VT32-10 mL - GERSTEL MultiPosition Vortexer (mVorx) - Anatune CF-200 Robotic Centrifuge
- *LC-UV:* Agilent 1260 Infinity Binary LC System, High Dynamic Range Infinity DAD Solution

Methods

Example 1: DHS-GC-MS for the analysis of Parkinson's disease skin swab samples

Samples were transferred into 20 mL headspace vials and then analysed by DHS-GC-MS.

Sample prep DHS: No purge Incubation: 5 min at 60°C Trapping: 500 mL at 50 ml/min, Trap 40°C No drying

GC-MS Runtime: 31 min

Example 2: Online MOX-TMS derivatisation of biological samples for untargeted metabolomics

Sample prep: Approximately 2 mg of freeze dried fungus was weighed into a 2mL high recovery vials. 100µL of MOX solution (16 mg/mL methoxyamine hydrochloride in pyridine) was firstly added to the sample and the mixture was incubated at 30°C for 90 min. Once methoximation was completed, 140µL of silylating reagent (MSTFA + 1% TMCS) was added to the sample and reacted for 30 minutes at 37°C. After centrifugation for 1 minutes at 4500 rpm, 2 µL of the top organic layer was directly injected on the GC-MS.

GC-MS Runtime: 45 min

Example 3: Online sample preparation for the analysis of tablets by LC-UV

One whole tablet was transferred to a 10 mL glass screw vial. 2 mL of water were added and the sample was then vortexed for 20 minutes. 2 mL of methanol and 1 mL of internal standard solution (benzophenone in methanol 40 mg/mL) were added to the same vial and the sample was further vortexed for 20 minutes. Once agitation was completed, the sample was centrifuged at 4500rpm for 5 minutes. An aliquot of supernatant solution was diluted to 10 mL with sample solvent and vortexed for 1 min at 3000 rpm to homogenize.

LC-UV Runtime: 20 min

Results and Discussion

Example 1: DHS-GC-MS for the analysis of Parkinson's disease skin swab samples

Dynamic Headspace (DHS) is a technique used to extract and concentrate volatile organic compounds from liquid or solid samples placed in standard headspace vials. The headspace above the sample is purged by nitrogen flow and analytes are concentrated on a user selectable adsorbent-filled trap. Analytes are subsequently introduced into the GC-MS by thermal desorption of the trap using a Thermal Desorption Unit (TDU). There are four main steps in a DHS method: incubation, purging, trapping and drying. Times for these steps need to be optimised depending on the specific application. The PrepAhead function enables overlapping of these four steps with the GC run of the previous sample reducing to a minimum downtime. Figure 2 shows the timelines for the analysis of 10 swab samples by DHS-GC-MS with and without PrepAhead. The multi-coloured bands represent the sample preparation and beige bands the GC run-time.

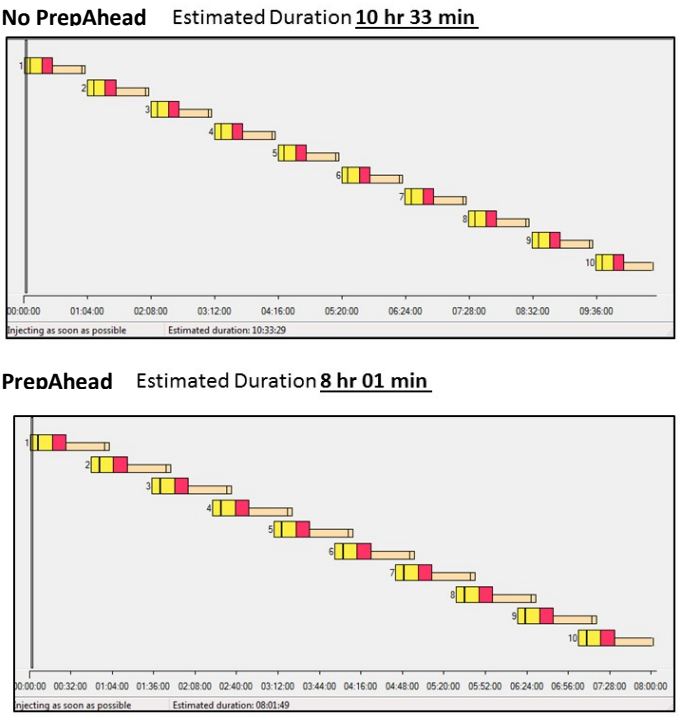

Figure 2: Timelines for the analysis of swab samples by DHS-TDU-GC-MS without (top) and with (bottom) Maestro PrepAhead function

The use of the PrepAhead function for the analysis of 10 skin swab samples by DHS-GC-MS allows to save approximately 2 hours and 30 min.

Example 2: Online MOX-TMS derivatisation GC-MS analysis of biological samples for untargeted metabolomics

Untargeted metabolomics carries out simultaneous measurement of a large number of metabolites from each sample providing the global metabolic profile. Since preparation of extensive sample sets is required to allow successful differentiation between sample types, analytical data quality is essential to highlight true biological variability. Methoximation followed by silylation (MOX-TMS), the most commonly adopted derivatisation method for GC-MS metabolomics. To avoid potential thermal degradation or species interconversion of metabolites, this dual step derivatisation is carried out at low temperatures (37°C) and to compensate the lower kinetic longer derivatisation times are required (2 hours per sample). As mentioned before, the PrepAhead function allows to perform the sample preparation for each sample immediately preceding the GC-MS injection whilst the previous sample is running. This not only offers a very time-effective option in a case like this where sample preparation is extremely lengthy eliminating

significantly operator downtime but also ensures that freshly derivatised samples are analysed promptly reducing the risk of degradation due to lifetime. Figure 3 showcases the extreme advantage when using PrepAhead function for this application.

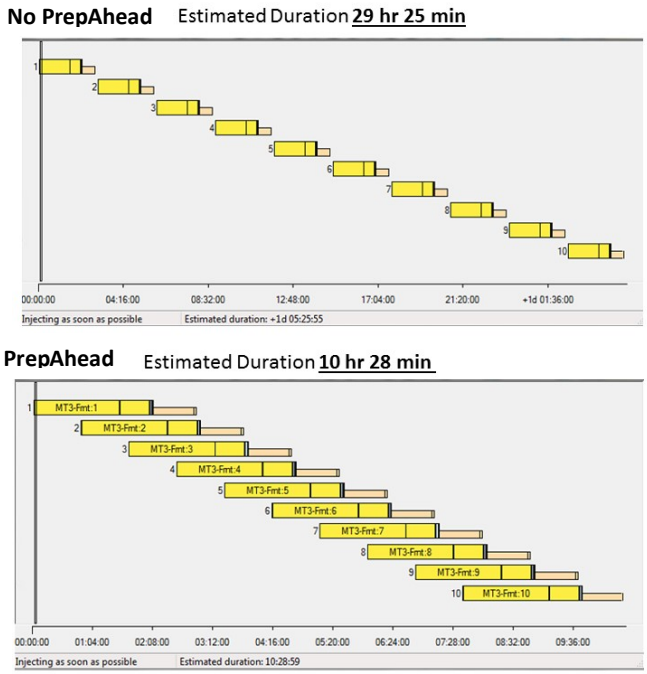

Figure 3: Timelines for the online MOX-TMS derivatisation and GC-MS analysis of biological samples without (top) and with (bottom) Maestro PrepAhead function

Preparation of ten samples without PrepAhead would require above 29 hours whilst using PrepAhead allows overlapping of a significant part of the sample preparation and GC runtime bringing down the duration for the same batch to just above 10 hours.

Example 3: Online sample preparation for the analysis of tablets by LC-UV

The benefits of the PrepAhead function are not limited to GC-MS applications. Sample preparation can be tedious and time consuming for LC applications as well. Figure 4 shows the timelines for the preparation and LC-UV analysis of ten tablet samples without (top) and with (bottom) Maestro PrepAhead. The use of PrepAhead consents to decrease the total batch duration from 9 hours and 31 minutes to 6 hours and 37 minutes.

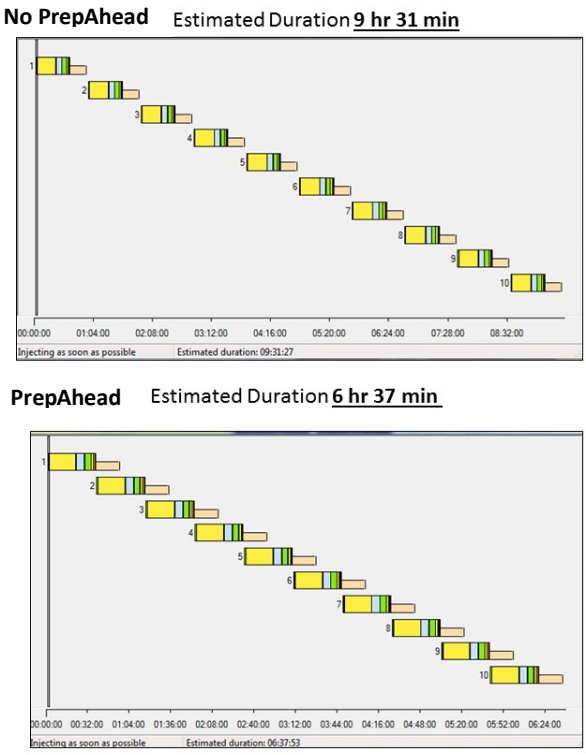

Figure 4: Timelines for the preparation and LC-UV analysis of tablets without (top) and with (bottom) Maestro PrepAhead function

Conclusions

GERSTEL Maestro PrepAhead function is an extremely useful feature which allows to perform the sample preparation for each sample immediately preceding the instrumental analysis (either GC or LC) whilst the previous sample is running. This not only offers a very time-effective option eliminating significantly operator downtime but also ensures samples to be prepared freshly every time before analysis reducing the risks of degradation and artifacts. This application note offered three examples of customer applications developed in our demo laboratory where the use of PrepAhead reduced considerably the total batch duration (in some cases up to 60%). If you would like a demonstration of how PrepAhead can help you please contact enquiries@anatune.co.uk.