Metabolite Profiling by Automated Methoximation and Silylation

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Overview

Purpose: Provide highly comparable data. Keep the sample integrity for metabolomics biomarker analysis by a highly reproducible and automated workflow for the two-step derivatization reactions used in GC-MS. Reduce the complexity of the manual operation and increase the sample throughput for unattended 24/7 operation.

Methods: A standardized automated workflow is suggested using a regular industry-standard x,y,z-robotic system for the individual derivatization steps at different reaction temperatures. The reagent addition, timing of the derivatization steps and analysis are controlled by the robotic system. The derivatization procedure follows the well-established and globally applied protocols.

Results: The automated derivatization workflow is executed in parallel to the chromatographic analysis by GC-MS. All samples are treated on the same time scale without waiting for analysis. The injection to GC-MS is executed right after completion of the derivatization steps.

Introduction

GC-MS analysis is extremely powerful for metabolite profiling due to the high chromatographic separation power of hundreds of compounds in one run. The GC separation of the mostly polar compounds by GC-MS requires a two-step derivatization procedure (aka MeOx-TMS derivatization) to reflect the in vivo state without any artefact formation [1]. The compound identification of the non-targeted metabolomics approach is facilitated by the many available huge mass spectra libraries as general or dedicated collections of well comparable standard spectra including also the typical silyl derivatives of the natural occurring compounds. Also, the GC-MS approach benefits generally from avoiding ion suppression from matrix effects, a major challenge faced in LC-MS analyses of complex samples.

The typical preparation procedure is a time-consuming manual operation. Most important for metabolite profiling is the identity and analytical reproducibility for each of the many compounds in a given biological matrix. Steps of the sample preparation include the frozen homogenization of the sample material with enzyme inactivation, adding internal standards, and fractionation into a polar and a lipophilic fraction. The polar fraction is dried and derivatized by first adding methoxyamine in pyridine, and then a trimethylsilylating agent like MSTFA or BSTFA before GC-MS analysis, finally followed by the data processing for statistical evaluations.

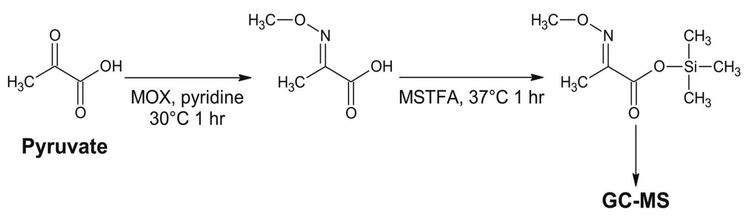

Figure 1. Two-step derivatization of pyruvate for GC-MS analysis [2].

The part of the two-step derivatization and following injection for GC-MS analysis is covered by the proposed automated workflow of a x,y,z-robotic system.

Methods

Scope and Principle of Operation

The first methoximation reaction (MeOx) protects carbonyl functions, as shown in Figure 1. Pyridine as solvent serves as a catalyst in the reaction. The following silylation step delivers the TMS derivatives of the polar compounds [1, 2].

For the MeOx reaction the samples are incubated with the methoxyamine solution in pyridine for 60 to 90 minutes at 28°C or 30°C. MSTFA or BSTFA with 1% TMCS as catalyst for silylation is then added to the mixture and incubated at 37°C for an additional 30 to 60 mins.

After completion of the silylation reaction, the vial is cooled down to RT in the sample rack before injecting into GC-MS for analysis.

The complete sequence of liquid handling steps, derivatization and incubation at different temperatures, finally the GC-MS injection is carried out in one comprehensive workflow as illustrated in Fig. 4 using the robotic sampling system.

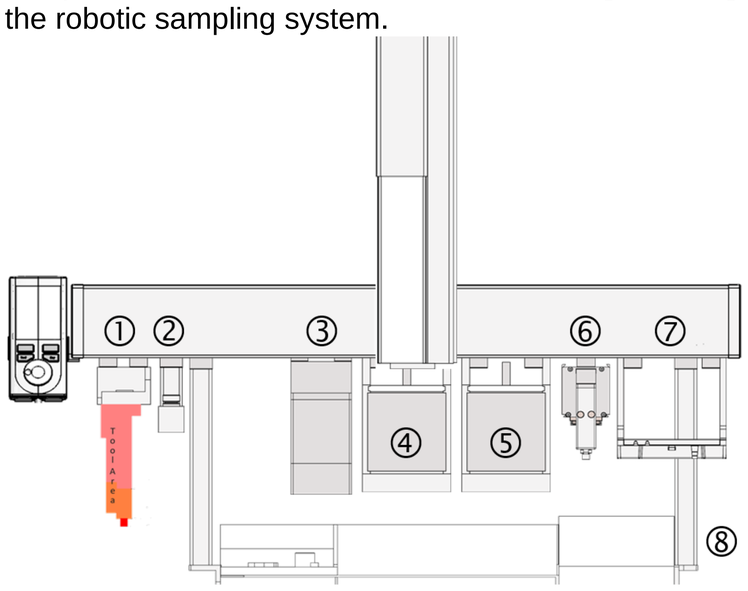

Figure 2. PAL x,y,z robotic system configured for the two-step derivatization workflow and online GC-MS injection.

1 Tool park station
2 Standard wash and reagent station
3 Vortex mixer
4 Agitator/Incubator 1
5 Agitator/Incubator 2
6 Fast wash station
7 Trayholder for sample vial racks
8 GC top mounting legs

System Configuration

For the workflow development a PAL RTC system was employed (CTC Analytics AG, Zwingen, CH). The configuration for the GC-top installation shown in Fig. 2 comprises two incubator modules set to the different derivatization temperatures. The vial transport is achieved by the use of magnetic vial caps.

The methoxyamine and silylation reagents are provided in dedicated positions of the wash/reagent station.

Variants

In case of using two dedicated syringes for derivatization and GC injection a tool park station for the exchange of the sample preparation syringe and GC injection syringe is used. The syringe tool change is then programmed before and after the GC injection with appropriate syringe wash cycles.

Also, if the same temperature for the MeOX and TMS derivatization is used, one incubator only is required.

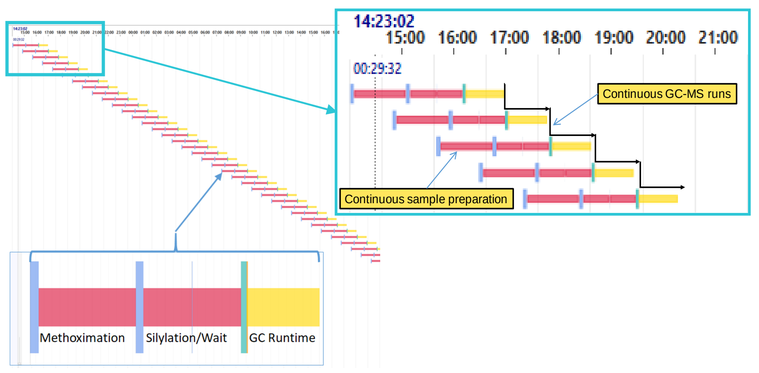

Figure 3. Continuous parallel derivatization and GC-MS analyses for high sample throughput.

Sample Throughput

Prep-Ahead Mode with Overlapping

Sample throughput is key for large sample series. The described workflow allows the derivatization of the next samples already during the GC-MS run of the previous sample. Important here is a feature that allows the PAL System to monitor the activity timeline and calculate the next sample preparation this way, that the next sample is ready at the expected end of the GC runtime and Ready signal.

The overlapping of the lasting derivatization steps as shown in Figure 3 keeps the sample throughput controlled by the total GC runtime only.

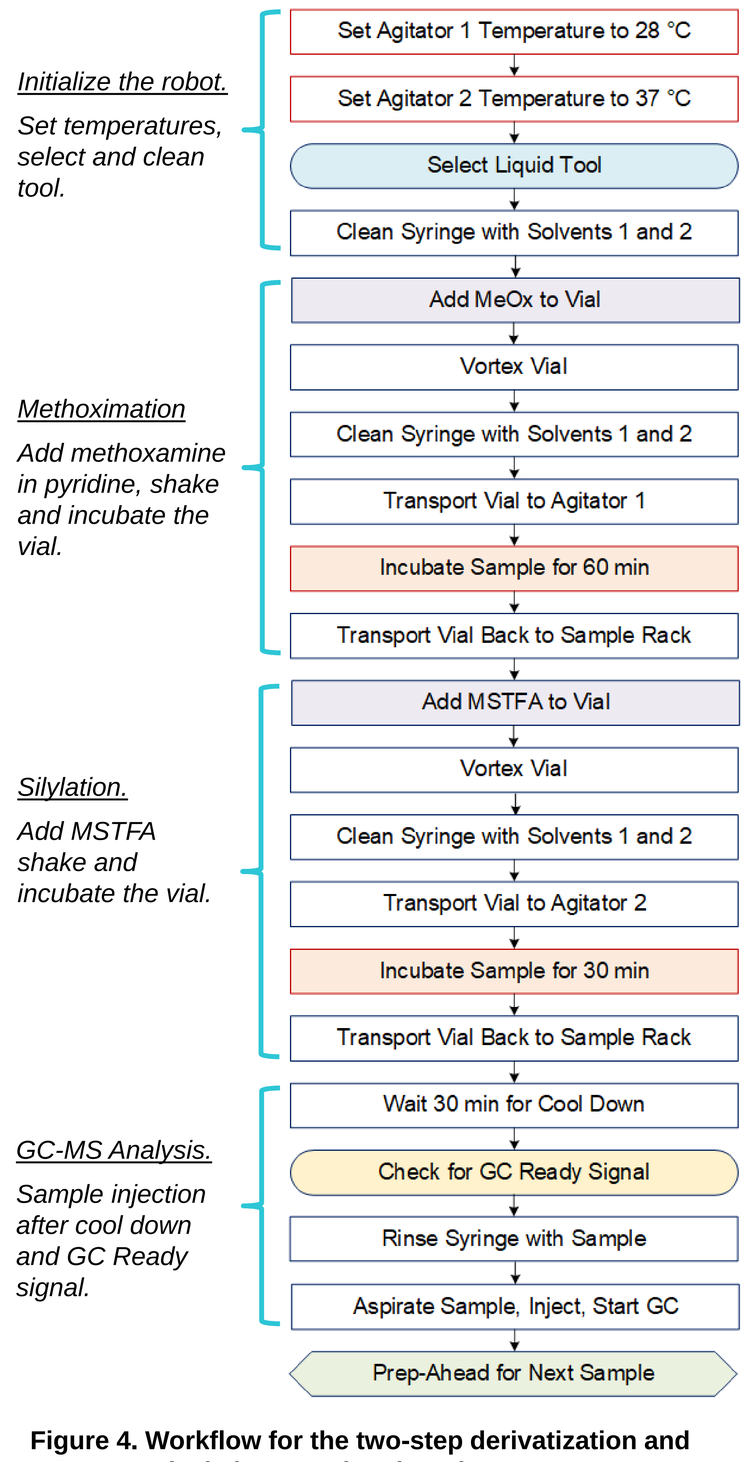

Figure 4. Workflow for the two-step derivatization and GC-MS analysis in prep-ahead mode.

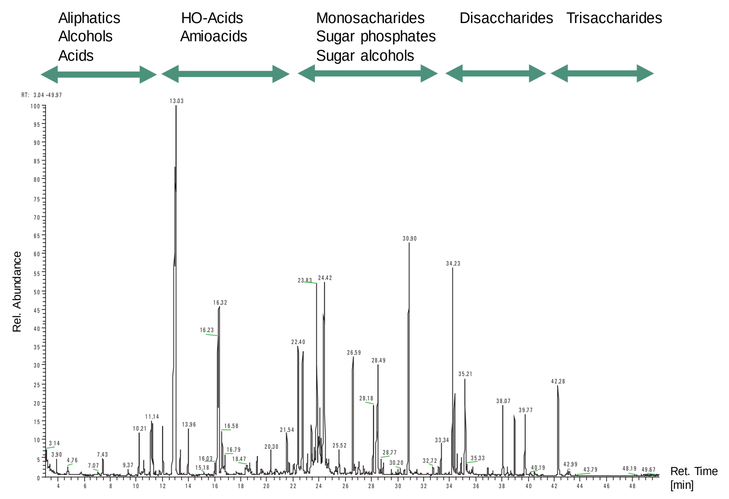

Figure 5. GC-MS full scan chromatogram after two-step derivatization, after [3].

Conclusion

A well described and accepted derivatization protocol is delivered with an automated workflow.

- The described workflow provides consistent sample preparation and data for comparison.
- All samples are treated with identical settings on the same time scale.
- The automated workflow allows the unattended 24/7 analyses of large sample series with online GC-MS analysis.
- A turnkey system and workflow for installation on every GC-MS system is available for rapid implementation.
- The results are independent on operator and location and warrant highly compatible data sets.

References

1. Fiehn, O.. 2005. “Metabolite Profiling in Arabidopsis.” In: Arabidopsis Protocols, Methods in Molecular Biology, Vol. 323, J. Salinas and J.J. Sanchez-Serrano (eds.), 439–47. Humana Press Inc.
2. Cox, J.E. et al. 2017. “Metabolomic Studies in Drosophila“. Genetics 206:1169-1185.
3. Fragner, L., W. Weckwerth, and H.-J. Huebschmann. 2012. “Metabolomics Strategies Using GC-MS/MS Technology”. Application Note 51999, Thermo Fisher Scientific, Austin TX, USA.