Metabolomics

Automated Sample Preparation Using the GERSTEL MPS Dual Head WorkStation

In metabolomics studies, relatively large sets of samples are processed to allow differentiation between sample types and the analytical variability must be lower than the biological variability. In order to achieve this, automating the sample preparation is a good first step, which can contribute significantly towards improving the repeatability of the total analytical procedure. Part 1of a series of 3 takes a closer look at Automated Ultrasonic Assisted Liquid Extraction and Filtration

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Metabolomics studies focus on the analysis of small molecules (MW<2000) in biological matrices from micro-organisms, plants, animals, and of human origin. Relatively large sets of samples are processed to allow differentiation between sample types and it is of course critically important to ensure that the analytical variability is lower than the biological variability. In order to achieve this, automating the sample preparation is a good first step, which can contribute significantly towards improving the repeatability of the total analytical procedure.

A typical metabolomics workflow includes extraction, fractionation or clean-up, derivatization, and a concentration step, followed by GC or LC separation and MS detection. In a series of articles, we describe a number of automated methods that are currently applied in our laboratories. In this first article, we focus on extraction and filtration. In a second article, an automatic fractionation procedure based on solid phase extraction will be described and in a final article, we will describe the use of an automated derivatization procedure prior to GC analysis.

For the extraction of plant material, ultrasonic assisted liquid extraction is a well-established method. However, ultrasonic extraction is mostly performed manually. This is in part due to the fact that solid particulates can create a suspension in the extraction solvent, which can easily block syringes, making automated collection of the extract and subsequent injection into the GC or LC unreliable. Applying recently introduced tools for the GERSTEL MultiPurpose Sampler (MPS), extraction, filtration and further processing of samples can be automated.

This is illustrated by an automated sample preparation protocol developed for the ultrasonic extraction of glycosides and phenolic compounds from plant material for a metabolomics study. The implementation of screen filters to prevent blockage of the MPS syringe along with 0.45 µm replaceable filter cartridges to filter the extract have enabled direct injection of the sonicated and filtered samples into an LC/MS system without the risk of system contamination with sample matrix.

Experimental

Automated Extraction

A 60 mg sample of ground plant material is weighed into a 10 mL headspace vial. Before capping the vial, a 17 µm stainless steel screen filter (GERSTEL p/n 020006-050-00) is placed inside the vial. Next, automated extraction and filtration is performed using an MPS Dual Head WorkStation (Figure 1). Extraction solvent (5.8 mL of 75/25 methanol/water) is added using a 2.5 mL syringe, followed by 0.2 mL internal standard solution using a 1.0 mL syringe. The vial is then transported by the MPS to the ultrasonic bath (Figure 2) and sonicated for 30 min. An aliquot (400 µL) of the extract is transferred from the sample vial (from inside the stainless steel screen filter) and filtered by the MPS using a disposable 0.45 µm filter cartridge (Figure 3). Figure 4 shows the sample vials before and after sample preparation. The MPS configuration is detailed in Table 1, and the MAESTRO Prep Sequence is described in Table 2.

Figure 1: MPS Dual Head WorkStation configured for automated ultrasonic extraction and filtration.

LC/MS

An Agilent Technologies 1290 Series UPLC System coupled to a 6540 Q-TOF LC/MS was used for the analysis of the extracts (Agilent Technologies, Waldbronn, Germany). A reversed-phase separation was performed on a C18 column using water, acetonitrile and formic acid as the mobile phase constituents.

Results and Discussion

For a metabolomics study of glycosides and phenolic compounds in plant material, 86 samples were prepared using the automated Prep Sequence described above. Of the 86 samples, 18 were quality control (QC) samples that were used to assess the reproducibility of the

Table 1: MPS Dual Head WorkStation Configuration.

MPS Module	Description
Left Arm	2.5 mL syringe with magnet for 10 mL vials
Right Arm	1.0 mL syringe with gripper
Tray and Holder	Ultrasonic bath with holder for 10 mL vials (6 positions)
Tray and Holder	10 mL headspace vials (VT-32)
Solvent Filling Station	Extraction solvent (75/25 Methanol/Water) + Wash (Methanol)
Tray and Holder	Filtration Tray (0.45 µm filters)
Tray and Holder	1.5 mL high recovery vials (VT-98) with filtration cover
Waste	Waste unit for used filters

Table 2: MAESTRO Prep Sequence used for Automated Sample Preparation.

Action	Arm	Description
Add	Left MPS	5,800 µL 75/25 Methanol/Water (Pre-rinse)
Add	Right MPS	200 µL IS (Pre-rinse Methanol)
Move	Left MPS	Tray 10 mL → Ultrasonic
Ultrasonic		30 min
Move	Left MPS	Ultrasonic → Dry*
Move	Left MPS	Dry* → Tray 10 mL
Get	Right MPS	Get filter (Gerstel # 017450-103-00)
Filtrate	Right MPS	400 µL + 600 µL air, Filter from above
Put	Right MPS	Transfer filter to the waste receptacle

*Dry is the name of the position in the ultrasonic tray, in which excess water is removed from the outside of the vials.

Table 3: Targeted analysis of the 18 QC samples.

Compound	Accurate Mass	tR (min)	%RSD Area
Rutin	610.1450	4.444	5.32
Chlorogenic acid	354.0950	3.319	5.95
Salicylic acid D5 (IS)*	142.0570	3.349	13.67
Kaempherol*	286.0480	7.197	13.60

*Low intensity.

Figure 3: Picture of the MPS picking up a 0.45 µm filter from the filtration tray.

Figure 4: Sample vials at the start (left) and end (right) of the automated sample preparation protocol.

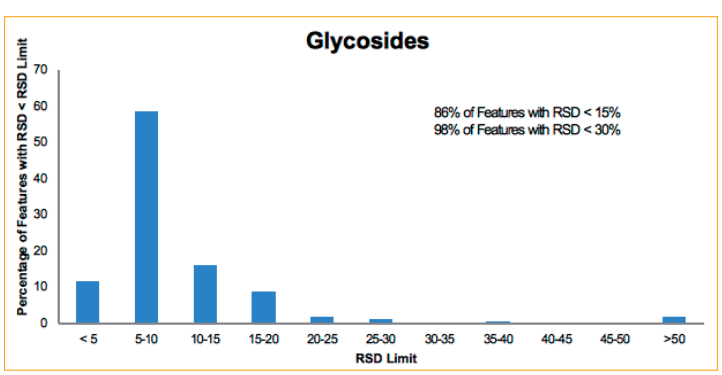


Figure 5: Bar plot showing the percentage of features with an area RSD value lower than the RSD limit (untargeted analysis).

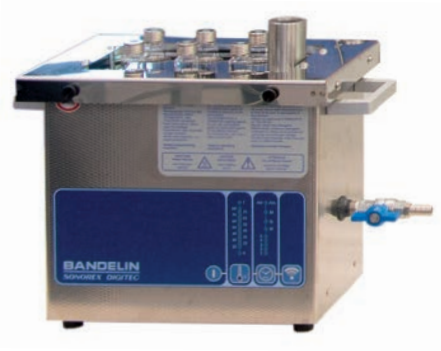


Figure 2: Ultrasonic bath option for the MPS WorkStation used for automated extraction of the plant material.

sample preparation and LC-MS protocol.

The combination of the consecutive filtration steps allowed unattended error-free sample preparation and injection sequences of 86 samples to be executed. The reusable stainless steel screen filter inside each sample vial prevents clogging of the MPS syringe needle by sample particulates dispersed in the extract. The liquid extract (methanol/water solution) was turbid after the ultrasonic agitation (see Figure 4) and additional filtration was needed. This was efficiently performed using the 0.45 µm disposable cartridge filters. Finally a clear extract was obtained that could be injected into the LC-QTOF system. All 86 samples were analyzed without any pressure increase on the 1290 UHPLC system.

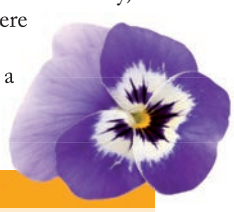
Both targeted and untargeted data analysis was performed on QC samples. For targeted analysis, the internal standard and a number of known compounds were selected and the area repeatability calculated; the results were excellent (Table 3). It should be noted that for metabolomics studies, the cutoff for area RSD values is typically 30 %. As can be seen from Table 3, the targeted analysis results obtained from the QC samples gave an area RSD of less than 14 % for the low intensity peaks, well within the limit for metabolomics data, and less than 6 % for Rutin and Chlorogenic acid.

For untargeted analysis, 590 features were considered. Plotting the area RSD values against RSD limits (Figure 5), it is clear that the results from the untargeted analysis were also excellent. As can be seen, 98 % of all features had area RSD values lower than 30 %, making them useful for further statistical evaluation.

Conclusions

The GERSTEL MPS Dual Head WorkStation is particularly useful for the automation of sample preparation in metabolomics studies. The combination of automated ultrasonic assisted liquid extraction and a dual filtration process results in extracts that can be analyzed directly by LC/MS. For the extraction of glycosides from plant material, in-vial stainless steel screen filters were successfully utilized to prevent blockage of the MPS syringe. Extracts were aspirated from inside the screen filter inserts, and taken through a further automated filtration step based on 4 mm 0.45 µm syringe filters before being injected into an LC/MS system. Following analysis of the quality control samples used in a metabolomics study, it was determined that the results obtained were highly repeatable.

In a following article, the automation of a SPE fractionation protocol applied in lipidomics will be described.



Glycosides

Glycosides are a class of compounds that contain a sugar and a non-carbohydrate moiety. The non-carbohydrate moiety is typically a small organic molecule. When the non-carbohydrate moiety is a phenol, the glycoside is for instance a flavonoid. In general, glycosides are secondary plant metabolites that are not involved in plant growth, development, or reproduction, but rather, in the interaction of a plant with the environment, such as UV protectants, pigment sources, and interactions with insects. In many plants, glycosides are important precursors of flavor-related compounds.