APPNOTE

GERSTEL
MAKING LABS WORK

GERSTEL AppNote 178

Automated Sample Preparation for Metabolomics Studies Using the Gerstel MPS Dual Head WorkStation - Part 1: Automated Ultrasonic Assisted Liquid Extraction and Filtration

Koen Sandra, Frank David, Christophe Devos, Bart Tienpont, and Pat Sandra

Research Institute for Chromatography, President Kennedypark 26, 8500 Kortrijk, Belgium

Abstract

In metabolomics studies, large sample sets have to be analyzed to allow statistical differentiation of sample types. Obviously, repeatability of the whole analytical workflow, including sample preparation, sample introduction, separation and detection, is extremely important in order to achieve such a differentiation. Automating the sample preparation workflow is a very useful first step towards reducing analytical variability.

In a series of articles, we will describe the use of the GERSTEL MPS WorkStation for automated sample preparation applied to metabolomics studies. In this first part, we highlight an automated sample preparation method, which was developed for the extraction of glycosides from plant material using the GERSTEL MPS Dual Head WorkStation. Ultrasonic assisted solvent extraction was performed on the plant material, and the extract was prepared for subsequent LC-MS analysis by two fully automated consecutive filtration steps, combining a first filtration on a 17 µm stainless steel screen filter placed in the sample vial and an additional filtration step using a 0.45 µm syringe filter. The obtained extracts were analyzed by LC-MS with excellent reproducibility.

Introduction

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) and sample preparation robot, 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 fil-

tered 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 a 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 SS 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.

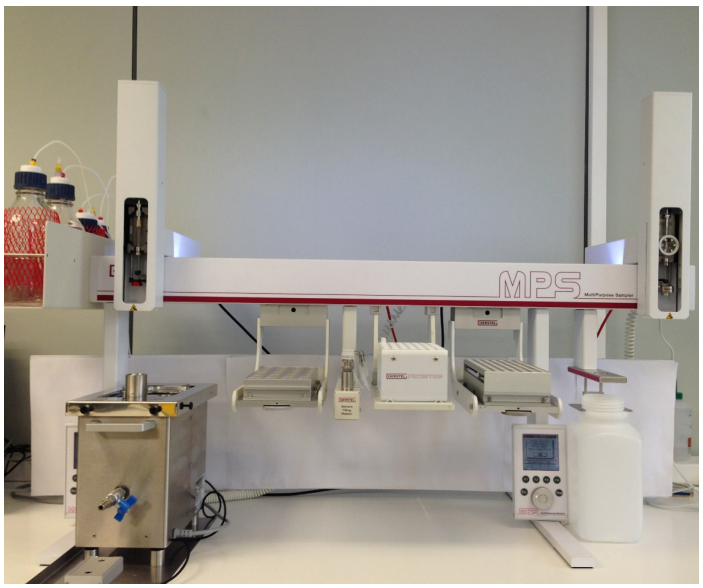

Figure 1: MPS Dual Head WorkStation configured for automated ultrasonic extraction and filtration.

Figure 2: Picture of the ultrasonic bath used for automated ultrasonic extraction of the plant material.

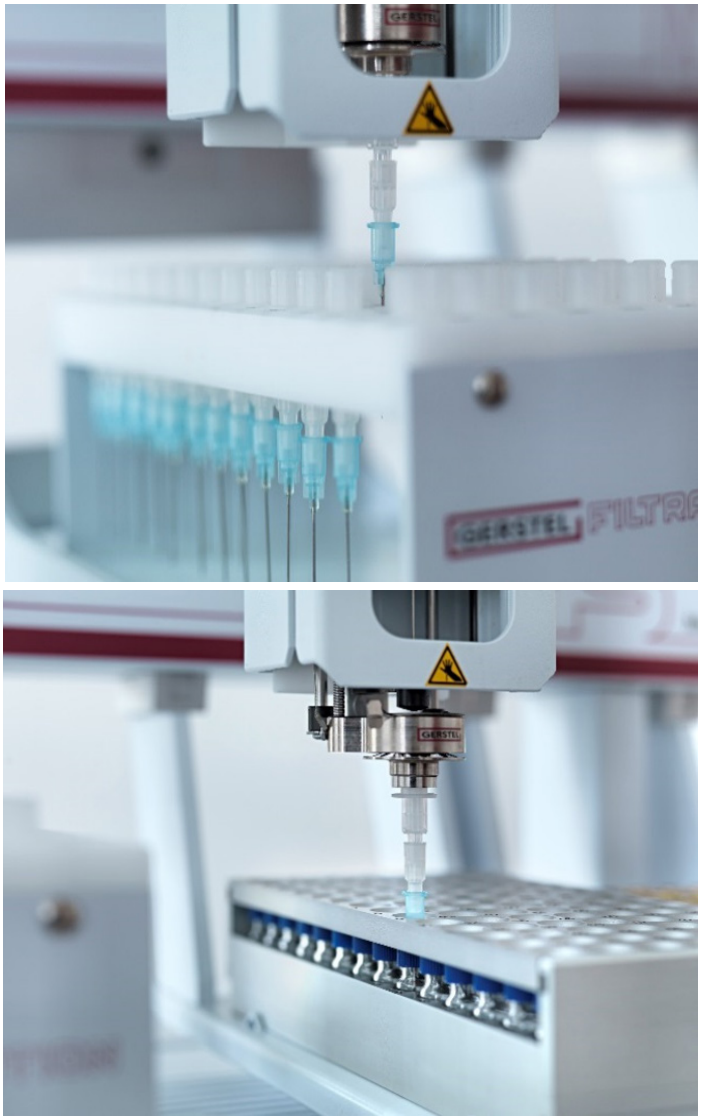

Figure 3: Pictures of the MPS transporting the 0.45 µm filter from the filtration tray (top) to the VT-98 tray with filtration cover (bottom).

GERSTEL AppNote 178

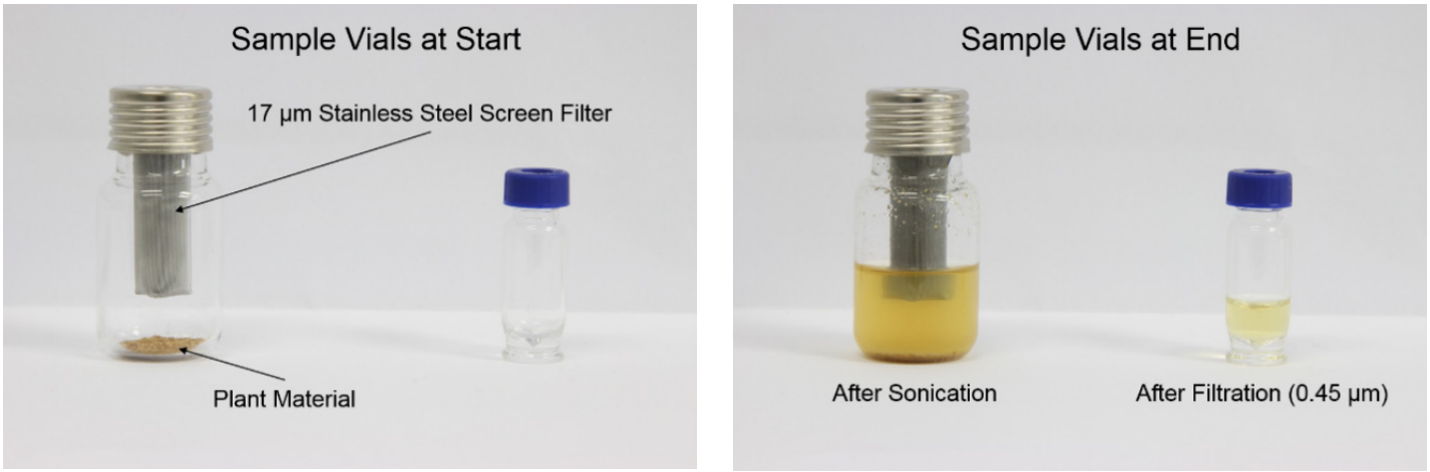

Figure 4: Sample vials at the start (left) and end (right) of the automated sample preparation protocol.

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 position name of the ultrasonic tray that removes excess water from the vials.

GERSTEL AppNote 178

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 sample preparation and LC-MS protocol.

The combination of the consecutive filtration step allowed an unattended error-free sample preparation and injection sequence of all 86 samples. The re-usable SS screen filters inside the sample vials prevent clogging of the MPS syringe needle due to sample particulates dispersed in the extract. The liquid extract (methanol/water solution) was cloudy after ultrasonic agitation (see Figure 4 right) and additional filtration was needed. This was efficiently performed using the 0.45 µm disposable cartridge filters. Finally a clear extract is obtained that can 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.

Table 3: Targeted analysis of the 18 QCs.

Compound	Accurate Mass	t_R (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.

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

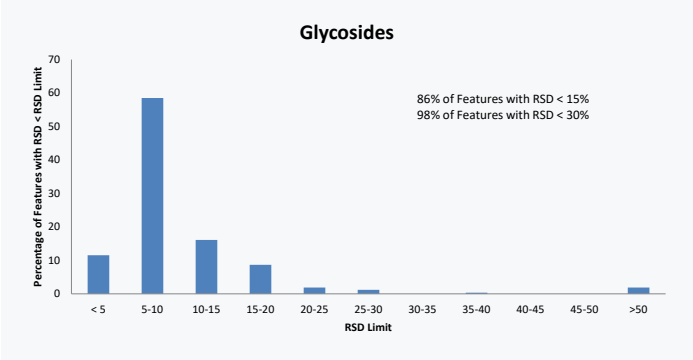

Figure 5: Bar plot showing the percentage of features with an area RSD value lower than the RSD limit (untargeted analysis).

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 by LC-MS. For the extraction of glycosides from plant material, in-vial SS 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.