AppNote 1/2015

Automated Sample Preparation for Metabolomics Studies Using the Gerstel MPS Dual Head WorkStation

Part 2: Automated Lipid Fractionation Using Solid Phase Extraction

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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 hereby of the utmost importance. In this respect, automation of the sample preparation is very useful in order to reduce the analytical variability.

In a series of articles, we describe the use of the Gerstel MPS WorkStation for automated sample preparation applied to metabolomics studies. In a first part, an automated ultrasonic assisted extraction and filtration method was discussed. In this second part, an automated fractionation of lipid classes using solid phase extraction (SPE) is presented. The SPE fractions are concentrated using an mVAP evaporation station and re-dissolved in small amounts of solvent, followed by LC-QTOF analysis.

Introduction

Metabolomics focuses on the analysis of small molecules (MW<2000) in biological matrices. Hereby relatively large sets of samples are processed to allow differentiation between sample types. In this respect, analytical variability should be much lower than biological variability and automation of sample preparation can significantly contribute to improved repeatability of the total analytical procedure.

In a typical metabolomics workflow, extraction of the sample is followed by fractionation or clean-up, if needed derivatization, concentration, and finally 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 a first article, automated ultrasonic assisted liquid extraction and filtration using the Gerstel MPS Workstation were discussed [1]. In this second article, an automatic fractionation procedure based on solid phase extraction (SPE) is described. This method was used in a lipidomics study, focusing on the characterization of plant material based on the relative composition of different classes of lipids, including neutral lipids (triglycerides, sterols), free fatty acids and polar lipids. Due to the fact that these classes are present in the plant material at substantially different concentration levels, it was observed that fractionation and selective enrichment of lipid classes prior to LC-MS analysis resulted in a much better coverage of lipids [2].

After liquid-liquid extraction, based on the Folch method [3], a concentrated lipid fraction was obtained. Next, fractionation was performed in a “normal phase LC” mode on an aminopropyl SPE cartridge. Three fractions of increasing polarity were obtained and the extracts were concentrated using an mVAP evaporation station installed on the MPS Workstation. Finally, the concentrated extracts were analysed by LC-QTOF.

Experimental

Automated Extraction. A one g sample of plant material was extracted with 6 mL chloroform:methanol (2:1). Next, 4 mL water was added and 1.5 mL from the bottom chloroform layer was filtered into a high recovery vial. The solvent was evaporated in an mVAP station. Extraction, filtration and concentration was performed on a separate MPS WorkStation unit.

Fractionation by Solid Phase extraction. Automated SPE and concentration were performed using a MPS Dual Head WorkStation configured as illustrated in Figure 1 and listed in Table 1.

Figure 1. MPS Dual Head WorkStation configured for automated SPE and concentration.

Table 1. MPS Dual Head WorkStation configured for automated SPE and concentration.

MPS Module	Description
Left Arm	500 µL syringe
Right Arm	2.5 mL syringe
Tray and Holder	10 mL headspace vials for SPE fractions
Tray and Holder	SPE cartridges
Wash Station	Needle wash
Stacked Tray	1.5 mL high recovery vials for filtered extracts (samples)
SPE Module	Perform SPE
mVAP	Vacuum assisted evaporation of extracts and SPE fractions
Solvent Filling Station	SPE Solvents – Hexane, 2:1 Chloroform/IPA, Diethyl ether (2% acetic acid), and MeOH

The extracts obtained from the extraction and filtration steps (2.1) were reconstituted in 300 µL chloroform. These extracts were fractionated using the SPE protocol shown in Figure 2. Basically three fractions of increasing polarity were obtained, containing neutral lipids (NLs), free fatty acids (FAs) and polar lipids, respectively.

These three fractions (collected in 10 mL vials) were concentrated to dryness in the mVAP station and reconstituted in chloroform:isopropanol for LC-MS analysis. Solvent amounts were optimized according to the concentration of the lipids in the extracts [2].

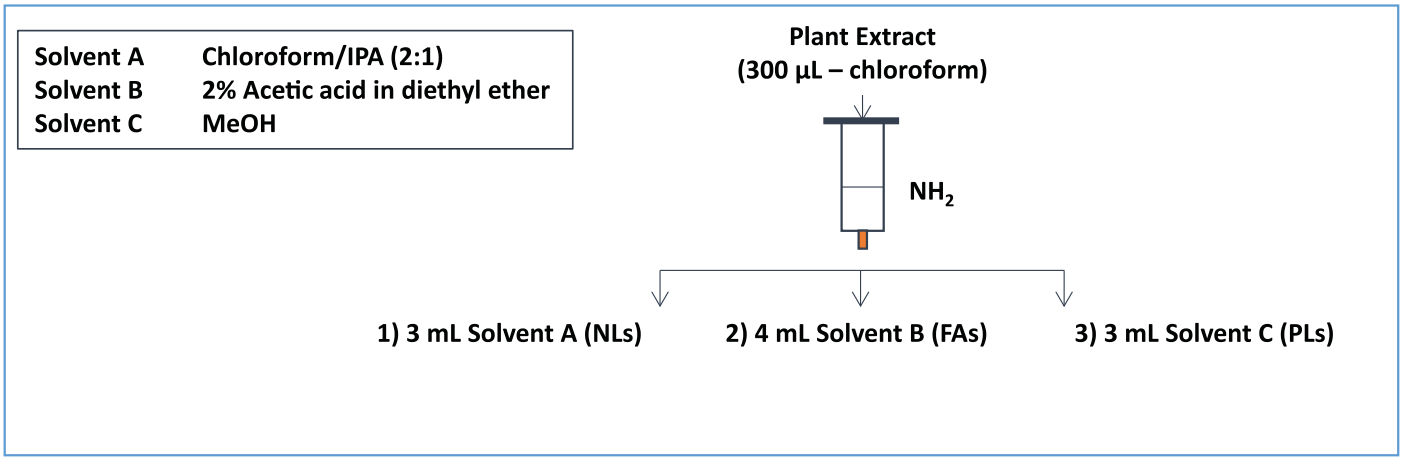

Figure 2. Automated SPE procedure.

LC-MS. An Agilent Technologies 1290 Series UHPLC 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 20 mM ammonium formate in water and methanol as the mobile phase constituents [4]. In total, 4 LC-MS methods were used, applying slightly different gradients and different MS conditions. Fraction 1 was analyzed using positive electrospray ionization (ESI POS), fraction 2 was analyzed in negative ESI mode (ESI NEG), and fraction 3 was analyzed both in ESI POS and ESI NEG modes

Results and Discussion

For a plant lipid study, 84 samples were prepared using the automated SPE method described above. Samples from 22 individual plants, belonging to 3 main types, were each prepared in triplicate. In addition, 18 quality control (QC) samples were analyzed to assess the reproducibility of the sample preparation and LC-MS protocol. Photos of reconstituted SPE fractions of three plant samples (each belonging to a different main class) are shown in Figure 3.

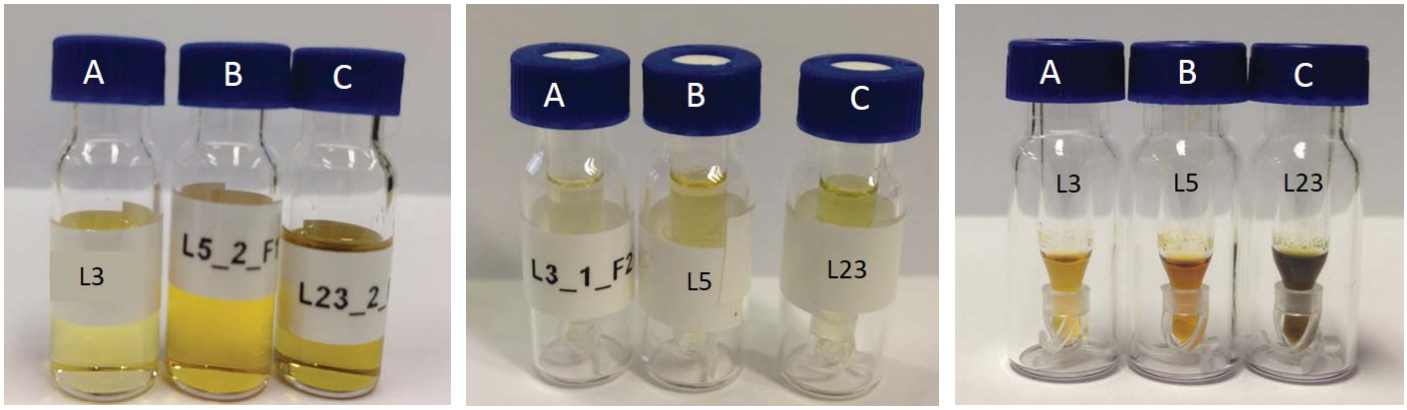

Figure 3. Reconstituted plant extracts after SPE fractionation and mVAP concentration (A, B and C correspond to three types of plants).

Typical LC-MS chromatograms are shown in Figure 4. The upper trace shows the analysis of fraction 1 (neutral lipids) in ESI POS mode. Monoglycerides (MGs), diglycerides (DGs), triglycerides (TGs) and plant sterols are detected. Trace B shows the analysis of the long chain free fatty acids (LCFAs) present in fraction 2 using ESI NEG mode. Traces C and D show the detection of phospholipids (PLs), sphingolipids and other polar lipids in respectively ESI POS and ESI NEG modes.

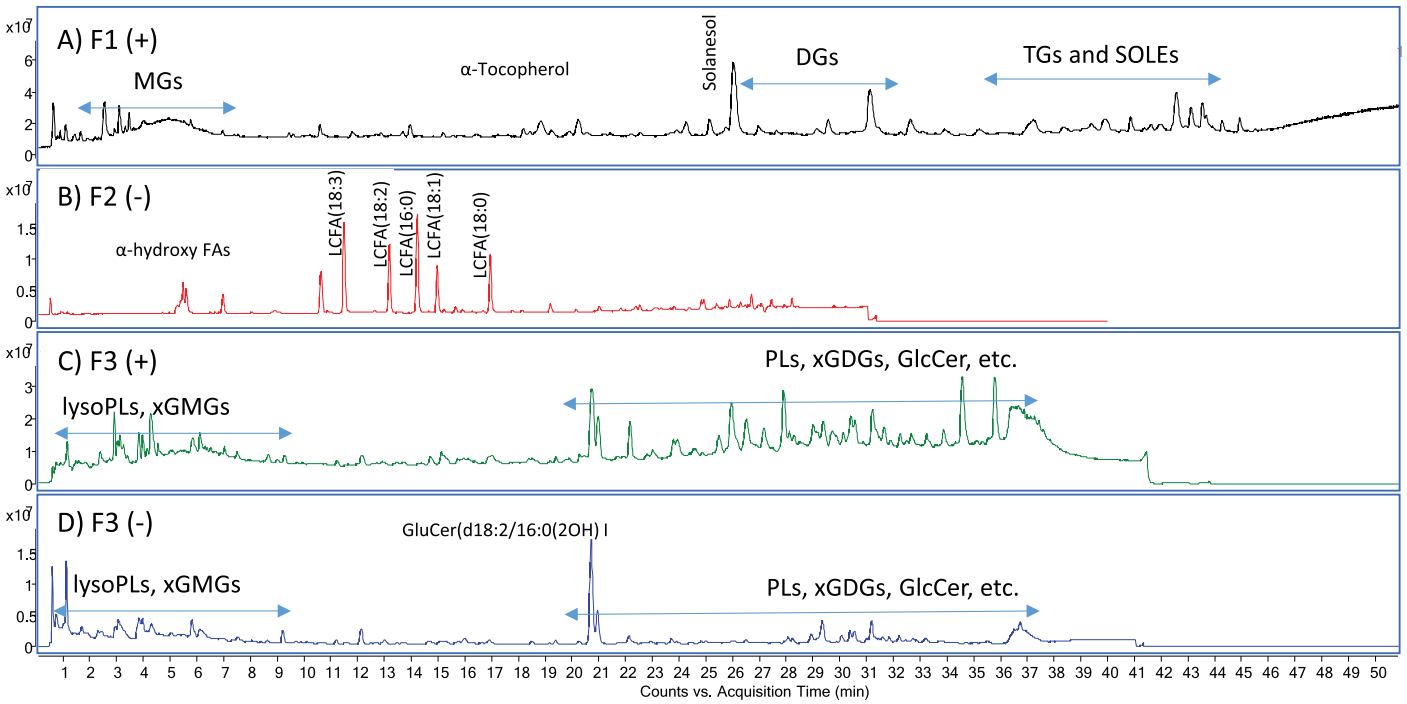

Figure 4. Total ion chromatograms of LC-QTOF analyses of the SPE fractions. A: fraction 1 in ESI POS mode, B: fraction 2 in ESI NEG mode, C: fraction 3 in ESI POS mode, D: fraction 3 in ESI NEG mode.

To evaluate the precision, a number of identified compounds were selected, and the area RSD% of each calculated. The results are presented in Table 3. It should be noted that for large-scale lipidomics studies, the cutoff for area RSD values is typically 30% [5]. As can be seen from Table 3, the results obtained for the 18 QC samples gave an area RSD of less than 20% in most cases.

Table 3. Precision of lipidomics methods including automated sample preparation.

Fraction	Lipid	Mass	TR (min)	%RSD Area
F1 (+)	MG(18:3)	369.2879	6.389	9.57
	solanesol	647.6005	29.097	8.61
	LANE(18:3)	703.6267	38.749	5.49
	SOLE(18:3)	907.8145	44.918	7.74
	MG(18:3)	369.2879	6.389	9.57
F2 (-)	LCFA-OH(18:3)	294.2210	7.540	11.66
	LCFA(18:3)	278.2259	14.497	5.38
	LCFA(16:0)	256.2414	17.245	6.16
F3 (+)	MGMG(18:3)	531.3407	8.137	22.12
	LysoPC(18:1)	521.3481	9.783	21.64
	GlcCer(d18:2/16:0)	697.5493	25.470	23.38
	PC(36:2)	785.5935	30.794	18.72
	MGDG(36:0)	803.6486	34.540	8.18
F3 (-)	MGMG(18:3)	560.3197	8.186	10.06
	LysoPC(18:1)	567.3550	9.868	19.36
	GlcCer(d18:2/16:0)	713.5471	24.494	15.24
	PC(36:2)	831.5980	30.745	10.33
	MGDG(36:0)	832.6212	32.410	5.77

Conclusions

The Gerstel MPS dual head WorkStation is particularly useful for the automation of sample preparation in metabolomics studies. A lipid class fractionation method based on solid phase extraction was fully automated on a dedicated configuration, including concentration of the SPE fractions by solvent evaporation. The LC-QTOF analysis results for the fractions showed excellent repeatability.
In a following article, the automation of a derivatization protocol applied in metabolomics will be described.

References

[1] Koen Sandra, Frank David, Christophe Devos, Bart Tienpont, and Pat Sandra, Automated Sample Preparation for Metabolomics Studies Using the Gerstel MPS Dual Head WorkStation, Part 1: Automated Ultrasonic Assisted Liquid Extraction and Filtration, Gerstel application note 8/2014.

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