Automated Sample Preparation Using the GERSTEL MPS WorkStation

Automated Lipid Fractionation Using Solid Phase Extraction

Metabolomics studies focus on the analysis of small molecules (MW<2000) in biological matrices, processing relatively large sets of samples to allow differentiation between sample types. In order to obtain statistically meaningful results, 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.

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In a typical metabolomics workflow, extraction of the sample is followed by fractionation or clean-up, and 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 the 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 a Multi-Position Evaporation Station (*m*VAP) installed on the MPS Workstation. Finally, the concentrated extracts were analyzed by LC-QTOF.

Lipidomics

Lipidomics is the large-scale study of pathways and networks of cellular lipids in biological systems. The word „lipidome" is used to describe the complete lipid profile within a cell, tissue or organism and is a subset of the „metabolome" which also includes the three other major classes of biological molecules: proteins/amino-acids, sugars and nucleic acids. Lipidomics is a relatively recent research field that has been driven by rapid advances in mass spectrometry (MS) and other analytical technologies, as well as computational methods, coupled with the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension and diabetes. This rapidly expanding field complements the huge progress made in genomics and proteomics, all of which constitute the family of systems biology.
Source: Wikipedia

Experimental

Automated Extraction.
A one gram 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 *m*VAP 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.

The extracts obtained from the extraction and filtration steps 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 (PLs),

Table 1: MPS Dual Head WorkStation configured for automated SPE and analyte 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	Performs SPE using replaceable, standard dimension packed bed cartridges
*m*VAP	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

Image: RIC

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

respectively. These three fractions (collected in 10 mL vials) were concentrated to dryness in the ^{m}VAP 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].

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.

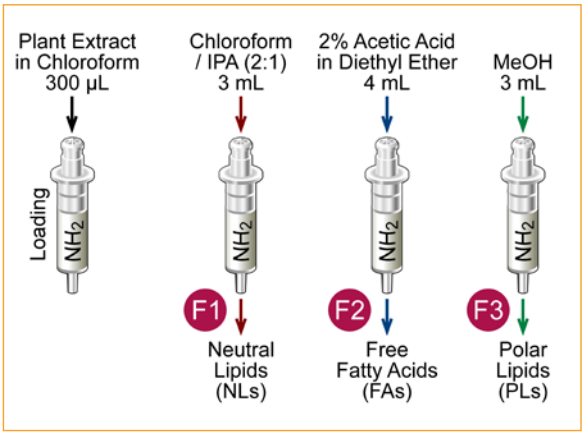

Figure 2. Automated SPE procedure

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.

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

Suggested reading

Development and validation of a robust automated analysis of plasma phospholipid fatty acids for metabolic phenotyping of large epidemiological studies.
Laura Yun Wang, Keith Summerhill, Carmen Rodriguez-Canas, Ian Mather, Pinal Patel, Michael Eiden, Stephen Young, Nita G Forouhi and Albert Koulman, Genome Medicine (2013) 5:39, DOI: 10.1186/gm443, http://www.gerstel.com/pdf/GST_GSW_10_20_22.pdf

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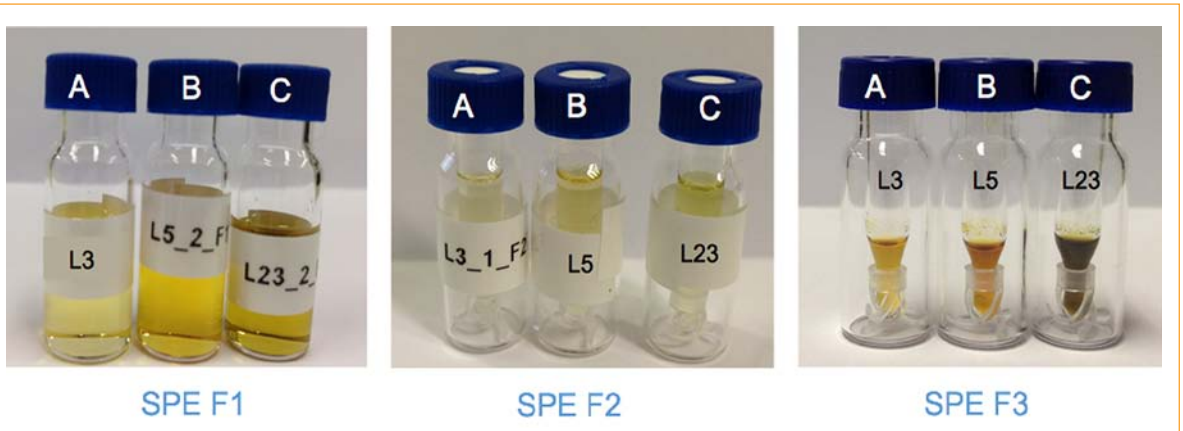

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Figure 3. Reconstituted plant extracts after SPE fractionation and ^{m}VAP concentration. A, B and C correspond to three different types of plants. F1: Neutral Lipids (NL); F2: Free Fatty Acids (FF); F3: Polar Lipids (PLs).

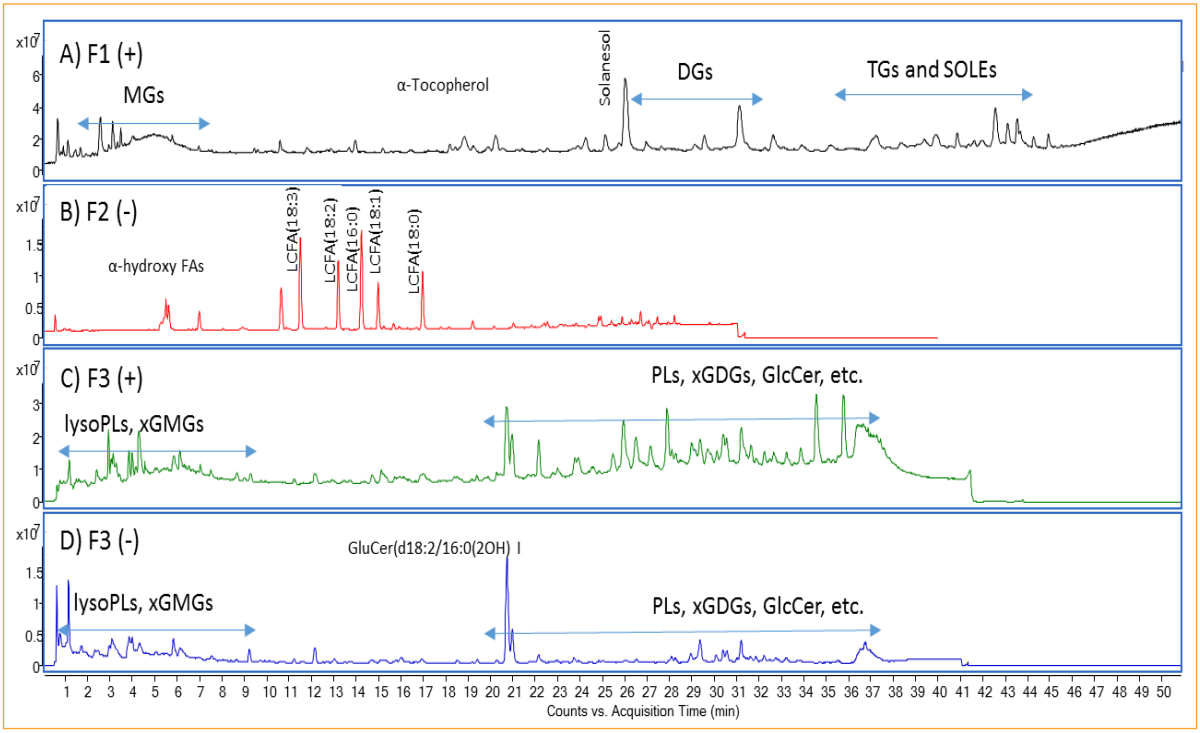

Image: RIC

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

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 ESI POS and ESI NEG modes respectively.

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.

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 platform, including concentration of the SPE fractions by solvent evaporation. The LC-QTOF analysis results for the fractions showed excellent repeatability. In an upcoming article, the automation of a derivatization protocol combined with GC/MS analysis applied in metabolomics will be described.

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

Fraction	Lipid	Mass	t_R [min]	% RSD Area
F1 (+)	MG (18:3)	369.2879	6.389	9.6
	Solanesol	647.6005	29.097	8.6
	LANE (18:3)	703.6267	38.749	5.5
	SOLE (18:3)	907.8145	44.918	7.7
F2 (-)	LCFA-OH (18:3)	294.2210	7,540	11.7
	LCFA (18:3)	278.2259	14.497	5.4
	LCFA (16:0)	256.2414	17.245	6.2
F3 (+)	MGMG (18:3)	531.3407	8.137	22.1
	LysoPC (18:1)	521.3481	9.783	21.6
	GlcCer (d18:2/16:0)	697.5493	25.470	23.4
	PC (36:2)	785.5935	30.794	18.7
	MGDG (36:0)	803.6486	34.540	8.2
F3 (-)	MGMG(18:3)	560.3197	8.186	10.1
	LysoPC(18:1)	567.3550	9.868	19.4
	GlcCer(d18:2/16:0)	713.5471	24.494	15.2
	PC(36:2)	831.5980	30.745	10.3
	MGDG(36:0)	832.6212	32.410	5.8

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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