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Metabolomics

Analytical Mass Movement

When epidemiological studies require analysis of large sets of blood, plasma, or urine samples under uniform conditions, with good accuracy, and within a limited time frame, manual sample preparation may not be the best approach. Metabolomics studies generally require efficient, fully automated sample preparation.

By Guido Deussing

According to experts, cardio vascular disease, obesity, Type 2 diabetes, and various types of cancer are often linked to a lack of exercise and to an unhealthy diet. Especially certain fatty foods can have a lasting influence on your health and well-being, but individual humans respond differently and are affected to different degrees. That, at least, is one way to explain why a fast food dominated diet causes some people to become overweight and increases their risk of developing diseases while others seem unaffected. The reason could be differences in genetic make-up with respect to metabolic processing of, and energy recovery from, nutrition in the organism.

In order to determine the effects of nutrition on health, as well as determining the role and influence of the gene pool, large epidemiological studies are undertaken that examine the resulting metabolites. The entirety of metabolites is referred to as the metabolome, the study and quantification of metabolites is referred to as metabolomics.

Profiling thousands of individual compounds

When searching for knowledge, in the form of correlations between health, genetics and nutrition, researchers use metabolite profiling hoping to find biomarkers that can provide information on metabolic processes in the organism. The tools they typically use are high level analytical instruments such as mass spectrometers, combined with gas chromatography (GC) or Ultra-High performance Liquid Chromatography (UHPLC), the combined instruments are typically referred to as GC/MS or LC/MS systems. In the case reported here, fatty acid methyl

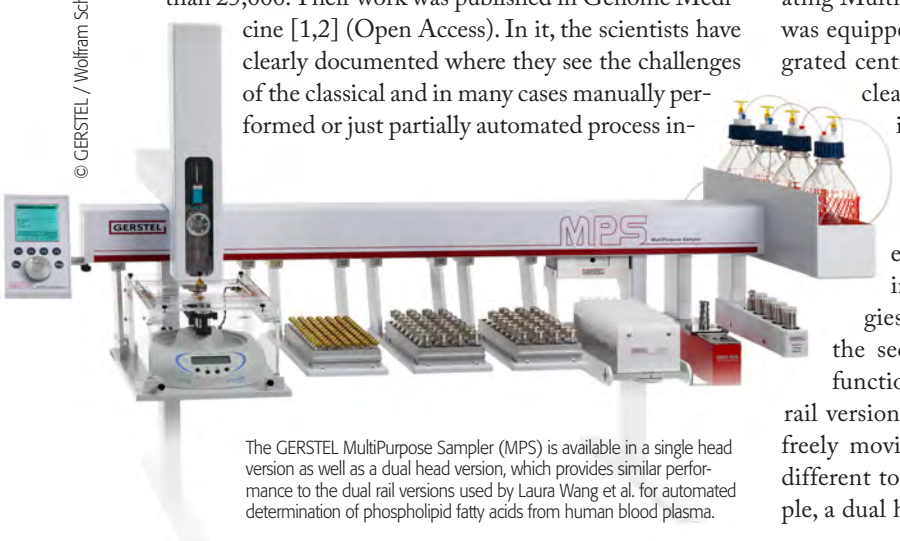
For quality control purposes, QC samples with a defined concentration of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were included and analyzed in every sequence. The table shows the median values in %. N is the number of sequences processed, SD the standard deviation, and % CV is the coefficient of variation in percent. The numbers demonstrate the excellent long term stability and reproducibility of the systems. [1]

Month	DHA			EPA		
	Mean	SD	% CV	Mean	SD	% CV
1 (n = 36)	2.14	0.06	2.60	0.46	0.02	4.22
2 (n = 36)	2.14	0.06	2.66	0.46	0.01	2.22
3 (n = 38)	2.13	0.06	3.02	0.47	0.03	7.28
4 (n = 38)	2.15	0.08	3.58	0.47	0.04	9.16
5 (n = 37)	2.16	0.06	2.66	0.47	0.03	7.13
6 (n = 38)	2.15	0.05	2.47	0.48	0.04	8.46
7 (n = 38)	2.16	0.04	1.78	0.47	0.03	6.54
8 (n = 38)	2.15	0.07	3.23	0.47	0.02	5.30
9 (n = 38)	2.15	0.06	2.71	0.46	0.03	6.90
10 (n = 38)	2.17	0.05	2.55	0.46	0.03	6.31
Comparison of mean values	$P = 0.062$			$P = 0.064$		

esters were quantitatively determined using a GC with flame ionization detector (GC/FID), due to the larger linear and dynamic range of the FID.

Metabolomics calls for automation

In order to obtain scientific evidence of possible health effects, you need large, comprehensive, and reliable data sets. It is not unusual for metabolomics studies to involve the determination of hundreds or even thousands of individual compounds. Just processing the large number of samples required within a reasonable time frame represents a challenge. In addition, it must be ensured that the results generated are extremely accurate in order to be able to draw any meaningful conclusions. To achieve all this, metabolomics experts agree, you cannot rely on manual sample preparation, you need as much automation as possible. As an example, Laura Yun Wang and her colleagues from the Elsie Widdowson Laboratories and the Institute of Metabolic Science in Cambridge, England, have reported on their work developing and validating a fully automated method for the determination of phospholipid-bound fatty acids in human blood plasma. The goal was to help perform metabolic phenotyping as part of a large epidemiologic study involving a sample set of more than 25,000. Their work was published in Genome Medicine [1,2] (Open Access). In it, the scientists have clearly documented where they see the challenges of the classical and in many cases manually performed or just partially automated process involved. They also report on how they have fully automated and validated the process steps needed.



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The GERSTEL MultiPurpose Sampler (MPS) is available in a single head version as well as a dual head version, which provides similar performance to the dual rail versions used by Laura Wang et al. for automated determination of phospholipid fatty acids from human blood plasma.

The Sample Preparation Challenge

The use of nutrition related biomarkers in large-scale epidemiological studies is only possible, "if the analysis is sufficiently fast, relatively cheap, robust and precise", according to Wang et al. It was decided to develop an automated method for fatty acid profiling of the phospholipid fraction in human plasma. A number of steps would have to be automated: The lipids would have to be extracted in total from the plasma using solid phase extraction (SPE) and converted into free fatty acids and then volatile fatty acid methyl esters (FAMEs), which can be determined using a GC with a flame ionization detector (FID). Using this method, stereo-isomers (cis- and trans fatty acid isomers) can be determined by GC in an acceptable time frame, according to the scientists.

When performed by hand, the large number of complex steps requires an inordinate amount of time and the process is prone to human error, as pointed out by Wang et al. As they found in their literature searches, multiple research groups had previously reported automated GC/FID analysis methods for fatty acid profiling in human plasma running a large number of samples. However, none had previously managed to automate the entire analysis, including extraction of the phospholipid fraction and hydrolysis and derivatization of the free fatty acids for a large set of samples as needed for epidemiological studies.

All Steps Completely Automated

Using a combination of three automated systems, the scientists succeeded in achieving the required high throughput combined with sufficient long-term stability and accuracy for the determination of phospholipid fatty acid fractions of human plasma samples. This was done as part of epidemiologic studies designed to correlate genetic and nutrition related factors with the development of type 2 diabetes. Each of the three automated systems consisted of two independently operating MultiPurpose Sampler (MPS) systems. One MPS was equipped with automated SPE option and an integrated centrifuge to perform automated extraction and cleanup of the lipid fraction. The second MPS in each system was integrated with the GC/FID and was used to perform sample preparation, i.e. the steps required for hydrolysis and derivatization to form fatty acid methyl esters (FAMEs) as well as introduction to the integrated GC 7890 N (Agilent Technologies, Little Falls, DE, U.S.A.). When choosing the second autosampler, Wang et al. focused on functionality. The MPS in the dual head or dual rail versions features two independently operating and freely moving towers that can simultaneously operate different tools, or syringes of different sizes. For example, a dual head system can simultaneously handle large

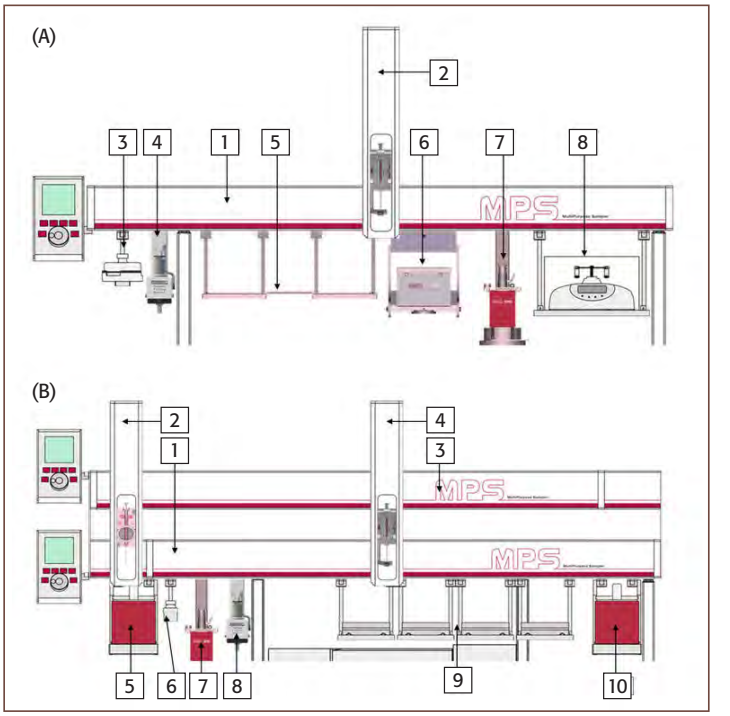
volume sample preparation and µL scale introduction to the GC analysis system. In order to achieve the required accuracy and reproducibility, reliable and accurate timing is necessary, especially when analyte derivatization is performed and the reaction product may not be stable. In such a case, the time period that elapses between analyte derivatization and introduction to the GC/FID system must be exactly the same for each sample in order to ensure accurate and reproducible results. In practice this means that samples are best prepared immediately prior to sample introduction. The MAESTRO software, which is used to operate the MPS systems by mouse-click automatically optimizes the process timing and ensures that every step is performed on time without having to be watched over by laboratory staff.

Final Words

Laura Yun Wang and her colleagues were confronted with a huge set of samples as part of the EPIC InterAct Project (See box below) for metabolic phenotyping of human plasma samples. Even with efficient automation, the analysis work took several months to complete. The three combined sample preparation and analysis setups used by the team performed extremely well over this extended period, delivering highly reproducible, reliable and stable results, including good instrument to instrument performance.

In direct comparison with the manual method, automation based on the MultiPurpose Sampler (MPS) came out ahead in many respects including improved standard deviation, as reported by Wang et al.; another aspect is the processing time per sample and the achieved throughput: Using the manual process, a team of two people could analyze 350 samples per month, or 4200 samples per year. Automating the process with three MPS systems operating in parallel enabled a team of four to process up to 90 samples plus standards and QC samples per day. On a monthly basis, 1,200 samples were analyzed, doubling the number of samples processed per person. Over a two-year period, 860 sequences were processed analyzing more than 25,000 samples in total [1]. The systems were described by the authors as both rugged and user-friendly, suitable for the determination of fatty acids in plasma phospholipids for both epidemiological research and routine analysis purposes. In addition, the method can easily be adapted to the analysis of other matrices such as cell extracts, tissue homogenates and food samples.

The work reported on in this article was part of a large case-cohort study of diabetes incidence nested within an even larger investigation into cancer and nutrition that includes 350,000 participants from 10 European countries. EPIC (European Prospective Investigation into Cancer & Nutrition) was designed to investigate the relationships between diet, nutritional status, lifestyle and environmental factors and the incidence of cancer and other chronic diseases.



MultiPurpose Sampler (MPS) Systems for automated sample preparation, derivatization of fatty acids from human plasma phospholipid fractions as well as sample introduction to GC/FID.
(A) MPS Single Rail for extraction of the phospholipid fraction, configured with: 1.) Solid phase extraction (SPE) unit; 2.) Syringe holder; 3.) Salt solution reservoir; 4.) Solvent reservoirs; 5.) Three tray holders; 6.) SPE cartridge tray; 7.) SPE / Evaporation; 8.) Vortexer / centrifuge.
(B) MPS DualRail/DualHead for hydrolysis, derivatization and injection of phospholipids 1.) Derivatization unit; 2.) Derivatization syringe holder; 3.) Injection unit; 4.) Injection syringe holder; 5.) Heated Zone; 6.) Wash bottles; 7.) SPE / Evaporation; 8.) Solvent reservoirs; 9.) Four tray holders; 10.) Agitator. [1]

InterAct

Investigating how our genes and lifestyle interact to lead to diabetes. The InterAct project started in 2006 and is an EU funded large scale collaboration between nine European Countries and India, designed to:

- discover how genetic and lifestyle behavioral factors, particularly diet and physical activity, interact in their influence on the risk of developing type 2 diabetes
- investigate how these discoveries may help to prevent the development of diabetes

More information: www.inter-act.eu

References

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