LIPIDOMIC PROFILING USING A PROTOTYPE MICROFLUIDIC MS PLATFORM
プロトタイプマイクロ流路デバイスを用いた脂質プロファイリング

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OVERVIEW

A prototype microfluidics platform for fast and robust lipidomics analyses with considerable reduction in solvent consumption and increase in sensitivity. Potential applications include large-scale lipid profiling and low-abundance lipids analyses in biological materials.

INTRODUCTION

Lipidomics is the comprehensive analysis of hundreds of lipid species in biological samples. Lipids play prominent roles in the physiological regulation of many key biological processes such as inflammation and neurotransmission. Alterations in lipid pathways have been associated with many diseases including cardiovascular diseases, obesity, and neurodegenerative disorders.

The ability to measure the wide array of lipid species in biological samples could help our understanding of their roles in health and disease. The need for a fast, comprehensive and sensitive analysis of the hundreds of lipid species challenges both the chromatographic separation and mass spectrometry.

Here we used a prototype microfluidics platform packed with 1.7 µm particles for fast and robust chromatographic separation. By integrating microscale LC components into a single platform design, the devices avoid problems associated with capillary connections and the need to keep the system free of leaks, blockages, and excessive dead volume. Such integrated microfluidic devices are suitable for lipidomics analyses with performance comparable to analytical scale LC-MS analysis.

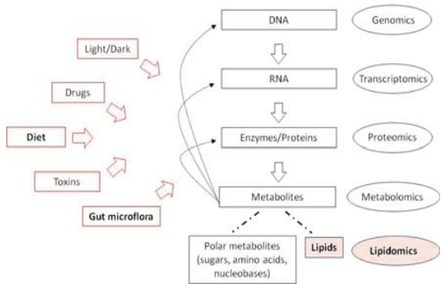

Figure 1. An overview of the –omics approaches currently used to analyze complex biological systems.

METHODS

Lipids were extracted from mouse plasma as previously reported [1,2] (Fig. 2). Analysis were conducted using prototype devices, which are fabricated from resistant ceramic materials that permit operation at high pressure with sub 2 micron particles. Lipids were separated using a nanoACQUITY UPLC engineered with 150 µm ID x 100 mm devices packed with C_{18} CSH (for untargeted analysis) and BEH (for targeted analysis) 1.7 µm particles. Mobile phases and analysis times were similar to regular LC methods using analytical-scale columns [1.2]. Flow rates were 2-3 µl/min. MS detection was conducted using a Synapt G2-S HDMS and a Xevo TQ-S operated in both negative and positive ES modes. TransOmics™ informatics solution and TargetLynx™ Application Manager were used to analyze the data.

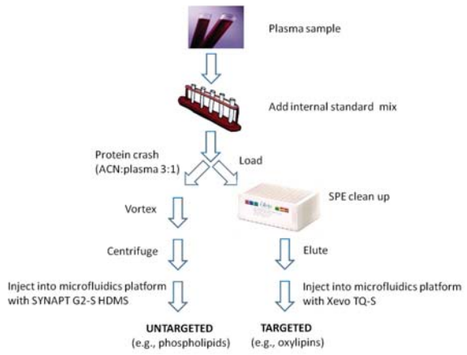

Figure 2. Workflow for the untargeted and targeted analyses of biological samples using the prototype microfluidics-MS platform.

UNTARGETED LIPIDOMICS

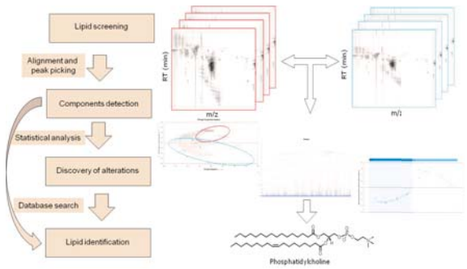

Figure 3. Untargeted lipidomics analysis of mouse plasma were conducted using TransOmics informatics, which allowed multivariate statistical analysis and database identification. Samples were analyzed using nanoACQUITY UPLC 150 µm device coupled with a Synapt G2-S HDMS[1].

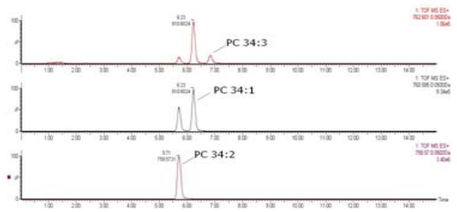

Figure 4. Representative extracted ion chromatograms of isobaric glycerophospholipids in mouse plasma. Samples were analyzed using nanoACQUITY UPLC 150 µm device coupled with a Synapt G2-S HDMS.

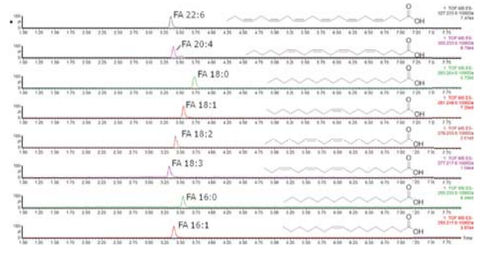

Figure 5. Representative extracted ion chromatograms of free fatty acids in mouse plasma using 150 µm ID x 100 mm devices packed with C_{18} CSH 1.7 µm particles. Lipids separated based on the number of double bonds and carbons.

	Microfluidics	Regular UPLC	
PC 14:0/14:0	0.15x100 CSH 18	2.1x100 CSH C18	
fmol on column	Area	Area	Fold Difference
36.85	540214	40504	11
18.42	328347	22217	15
9.21	146452	14395	10
4.61	83265	5233	16
2.30	40832	2823	14
1.15	19403	1287	15
0.58	8512	701	12
0.29	4333	377	11
0.14	2108	236	9
0.07	839	98	9
0.04	429	114	NA

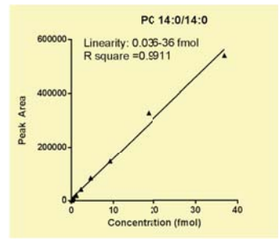

Figure 6. Analysis of a phosphatidylcholine molecule (PC 14:0/14:0) using either the prototype microfluidic-MS device or a regular ACQUITY UPLC system coupled with a Synapt G2-S HDMS operated in MS^E mode. Volume injected was the same on both systems (i.e., 0.2 µl).

TARGETED LIPIDOMICS

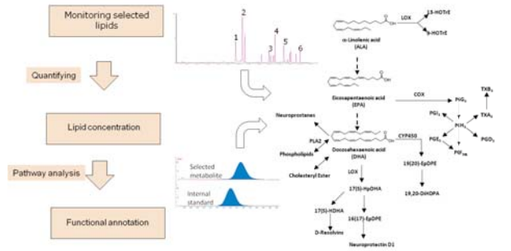

Figure 7. Targeted lipidomics analysis of mouse plasma using internal standards and TargetLynx for the identification and quantification of selected lipid molecules [2].

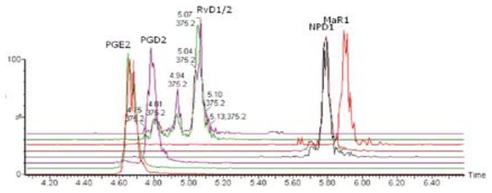

Figure 8. MRM extracted ion chromatograms of oxylipins species show separation of isomeric species such as PGE2 and PGD2. Samples were analyzed using nanoACQUITY UPLC with BEH 150 µm ID x 100 mm, 1.7 µm particles coupled with a Xevo TQ-S . Abbreviations: PG, prostaglandin; Rv, resolvin; NP, neuroprotectin; Ma, maresin.

CONCLUSIONS

The prototype microfluidics-MS platform lead to highly efficient LC separation of lipid molecules.

Chromatographic results were equivalent to using analytical-scale columns [1,2], bringing considerable advantages:

- **>200x decrease in solvent consumption, making it convenient for the large-scale analysis and screenings of hundreds or thousands samples.**
- **>10x increase in sensitivity, which could facilitate the detection of low abundance metabolites.**
- **low volumes injection (e.g., 0.2 µl), which makes it ideal when sample limited studies or when multiple injections are required.**

References

1. Isaac G, McDonald S, and Astarita G. "Lipid Separation using UPLC with Charged Surface Hybrid Technology". Waters App note. 2011. 720004107en.
2. Strassburg K, et al., "Targeted lipidomics of oxylipins (oxygenated fatty acids)". Waters App note. 2013. 720004664en.

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