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A not-stop-flow online normal-/reversed-phase two-dimensional liquid chromatography–quadrupole time-of-flight mass spectrometry method for comprehensive lipid profiling of human plasma from atherosclerosis patients☆

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ABSTRACT

A not-stop-flow online two-dimensional (2D) liquid chromatography (LC) method was developed for comprehensive lipid profiling by coupling normal- and reversed-phase LC with quadrupole time-of-flight mass spectrometry (QToF-MS), which was then applied to separate and identify the lipid species in plasma, making its merits in quality and quantity of the detection of lipids. Total 540 endogenous lipid species from 17 classes were determined in human plasma, and the differences in lipid metabolism products in human plasma between atherosclerosis patients and control subjects were explored in detail. The limit of detections (LODs) of 19 validation standards could all reach ng/mL magnitude, and the RSDs of peak area and retention time ranged 0.4–8.0% and 0.010–0.47%, respectively. In addition, a pair of isomers, galactosylceramides (GalC) and glucosylceramides (GluC), was successfully separated, showing that only the levels of GalC in atherosclerosis patients were significantly increasing, rather than GluC, compared with the controls (controls vs. patients: the ratio was 1.5–2.8-fold increasing). It would be helpful to the further research of the atherosclerosis.

1. Introduction

Lipids, defined as a wide variety of hydrophobic or amphipathic small molecules, play multiple and critical roles in numerous cellular functions and physiological processes [1–3]. It was known that some lipids are messengers in cell signaling transduction processes and can be utilized as biomarkers of some diseases [4–6]. Atherosclerosis is a chronic inflammatory disease characterized by dyslipidemia and abnormal lipid accumulation in the arterial intima, with activation of both innate and adaptive immunity [7,8]. Lipids were not only related to atherosclerotic plaque accumulation directly, but also linked to immune responses that cause cytokines release and macrophage and lymphocyte infiltration [9,10]. As lipid profiling aims at quantifying different kinds of lipids to investigate and understand their implication in a physio- or patho-physiological pathway of clinical interest, a growing interest has been paid in the study of atherosclerosis. However, the comprehensive lipid profiling is still demanding due to the complexity of the lipids.

As lipids can be divided into eight categories (fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides) containing distinct classes and subclasses [11], and each class of lipids contains a series of molecules with different combination of acyl chains, the simultaneous analysis of the entire lipidome presents a challenge in the analytical area. Recently, the direct infusion mass spectrometry (MS) is one of the major strategies of comprehensive lipids profiling [12]. However, the ion suppression effects among lipids, due to the lack of chromatographic separation, decrease the detection sensitivity, especially for low-abundance lipid species. Besides, discrimination of isomers of lipids is always a challenge for the direct infusion ESI-MS method [13]. As the development of liquid chromatography–mass spectrometry/mass spectrometry

☆ Selected papers contributed to the honor issue of Professor Peichang Lu's 90th birthday.

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Table 1
Configuration of online 2D LC–MS systems for lipid profiling.

2D LC system									
	1st D LC				Interface			2nd D LC	
T (min)	Flow (mL/min)	A1 (%)	B1 (%)	C1 (%)	Loop/position	*T*/min	Flow (mL/min)	A2 (%)	B2 (%)
0	0.2	80	20	0	1	0	0.3	90	10
5	0.2	80	20	0	2	5	0.3	90	10
						7	0.3	100	0
						14	0.3	100	0
						14.01	0.3	70	30
					1	19.50	0.3	70	30
						26.50	0.3	100	0
						33.50	0.3	100	0
						33.51	0.3	70	30
35	0.2	50	50	0					
					2	39.50	0.3	70	30
						44.50	0.3	70	30
						54.50	0.3	100	0
55	0.2	30	70	0					
						64	0.3	100	0
						64.01	0.3	70	30
65	0.2	30	70	0					
					1	70	0.3	70	30
75	0.2	20	80	0		75	0.3	70	30
						82	0.3	100	0
						88.5	0.3	100	0
						88.51	0.3	70	30
					2	94.50	0.3	70	30
						99.50	0.3	100	0
100	0.2	0	60	40					
100.01	0.2	0	0	100					
						106.50	0.3	100	0
						106.51	0.3	70	30
					1	112.50	0.3	70	30
						117.50	0.3	100	0
						123.00	0.3	100	0
						123.01	0.3	70	30
					2	129	0.3	70	30
130	0.2	0	0	100					
						134	0.3	100	0
						149	0.3	100	0

(LC–MS/MS) for lipidomic analysis, the range of lipids determination was greatly enlarged. Normal-phase (NP) LC and reversed-phase (RP) LC have both been used for different purposes in lipidomics analysis: the NPLC method is regularly used to separate different classes of lipids based on the polar head groups and the RPLC method is often used to separate different molecular species in one class based on the different fatty-acyl chains. Therefore, compared to other methods, online normal-phase/reversed-phase two-dimensional (NP/RP 2D) LC–MS method has significant advantages in chromatography separation [14]. In a previous work, online stop-flow NP/RP 2D LC–MS method was developed by introducing a solvent evaporation interface between NP and RP columns to remove the organic mobile phase used in the NPLC process [15]. Besides the combination of powerful NP and RP separation, the interface brings an enrichment of lipids by evaporation of the solvent and re-injection. However, this method also has some limitations. Due to the stop-flow of first dimension NPLC, the total analysis time was rather long, and peak broadening was obvious, which may affect the repeatability and sensitivity. Moreover, this work only focused on the capability of phospholipid identification, the application to a wide variety of lipid classes is still required.

In this article, we set up an automatic not-stop-flow NP/RP 2D LC system, by using a ten-port, two-position valve, coupled with quadrupole time-of-flight mass spectrometry (QToF-MS) for lipid profiling. This not-stop-flow method suppressed the sample peak broadening in the first dimensional column, so that the sensitivity and repeatability of the 2D LC method were increased. As a not-stop-flow method, the analysis time was decided only by the first dimensional separation, which could reduce the time-consuming of 2D LC method. In addition, a much wider variety of lipid classes were analyzed simultaneously by using this method, including FFA, glycerophosphoinositols (PI), glycerophosphoglycerols (PG), lysoglycerophosphoglycerols (LPG), glycerophosphoethanolamines (PE), lysoglycerophosphoethanolamines (LPE), glycerophosphoserines (PS), glycerophosphocholines (PC), lysoglycerophosphocholines (LPC), phosphosphingolipids (SM), ceramides (Cer), galactosylceramides (GalC), glucosylceramides (GluC), lactosylceramides (LacC), monoacylglycerols (MG), diacylglycerols (DG), and triacylglycerols (TG). Finally, this technique was implicated into the research between atherosclerosis patients and healthy volunteers for lipidomic analysis, firstly showing that only the levels of GalC in atherosclerosis patients were significantly increasing, rather than GluC, compared with the controls (controls vs. patients: the ratio was 1.5–2.8-fold increasing), which would be helpful for the further study of the atherosclerosis.

2. Materials and methods

2.1. Subjects

The study was approved by the Institutional Review Board and the Ethic Committee of Peking University First Hospital, Beijing, China, and subjects were provided written informed consent. The inclusion criteria were defined as: 1) subjects of 40–75 years of age with carotid artery atherosclerosis proved by ultrasound and 2) subjects under therapy of stroke prevention management, targeted on blood pressure and items of lipids metabolism in