A Novel analytical method to analyze Phosphatidylcholine in human breath using UHPLC-MS/MS.

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Introduction

Pulmonary surfactant is an important component of respiratory system consists of a complex mixture of phospholipids and proteins. Phospholipids constitute about 80 to 90% of pulmonary surfactant with rest comprises the pulmonary proteins. The primary function of this surfactant is to reduce the surface tension at air-liquid interface to prevent the collapse of the alveoli during expiration. Phosphatidylcholine (PC), an important component of surfactant comprises approximately 70 to 80% among phospholipids. Dipalmitoylphosphatidylcholine (DPPC) is the most abundant form comprised approximately 50 to 70% of the PC, and is believed to be the most important pulmonary surfactant to reduce the surface tension at the air-liquid interface. This study describes a LC-MS method development to analyze PCs in exhaled breath

Methodology

Particles in Exhaled breath was collected in a commercial sampling device (Sensabues AB, Huddinge, Sweden). A total of 25 L exhaled air pass through the device. During sampling of exhaled breath containing microparticles pass through a mouth-piece and subsequently collected to a polymer filter with a diameter of 30 mm inside the device. Afterwards, 5 ml MeOH was used to extract the analyte from the filter. The extracts were evaporated under ambient temperature and reconstituted in 100 µL of MeOH. Samples were analyzed by HPLC-MS/MS using a Dionex Ultimate 3000 UHPLC system coupled to a Thermo Fisher Scientific TSQ Quantiva triple quadropole mass spectrometer. The chromatographic separation was achieved on an acquity UPLC BEH phenyl column (2.1 × 50 mm, 1.7 µm) with injection volume of 2 µL at a mobile phase flow rate 400 µl/min in a gradient mode. Mobile phase A consisting of 95:5 water:methanol and a mobile phase B consisting of 95:5 MeOH: water, with 4 mM ammonium formate and 0.1% ammonia in both A and B. The

gradient profile was as follows: starting with 50% B (hold time 0.3 min) and continued with linear change to 90% B upto 1 min and to 100% B up to 1.2 min. Continued 100% B up to 5.8 min and returned to initial condition at 5.9 min followed by equilibration until 7 min. The mass spectrometer was operated in electrospray ionization in positive ion mode. The MRM transitions for PC 32:0 and PC 16:0/18:1, were m/z 734.5>184 and m/z 760.5>184, respectively.

Results

During chromatographic optimization, variety of analytical columns was tested. Excellent chromatographic separation was achieved with the column mentioned above. The method detection limits (MDLs) were 2 and 5 pg/filter for PC 32:0 and PC 16:0/18:1, respectively. The whole method linearity was assessed by making a 6 point standard calibration curve with native analytes spiking the Sensabues filter with concentrations ranging from MLQ to 10 ng/filter. Excellent method linearity was achieved ($r^2 \geq 0.99$) for both analytes indicating that the recoveries were not concentration dependent within the concentration range. Other validation parameters including recovery, accuracy and repeatability were achieved with satisfactory performance.

Conclusions

In this study, we successfully identify and quantified the PCs in exhaled breath. This is the first study where we developed a method to identify the phosphatidylcholine in human breath which PC 32:0 was predominantly found than PC 16:0/18:1.