ANALYSES OF FAT-SOLUBLE VITAMINS, CAROTENOIDS AND LIPIDS BY SUPERCRITICAL FLUID CHROMATOGRAPHY WITH SUB-2 µm PARTICLE COLUMNS

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INTRODUCTION

UltraPerformance Convergence Chromatography™ (UPC²) is a separation technique that uses compressed carbon dioxide as the primary component of the mobile phase. It takes advantage of the unique physical properties of compressed carbon dioxide (at or near supercritical state), sub-two micron particle chromatography columns and advanced chromatography system design to achieve fast and reproducible separation with high efficiencies and unique selectivity.

These improvements lead to new interest in applying this technology to various industrial analytical areas, especially those areas where normal-phase liquid chromatography (NPLC) has been commonly used, such as fat-soluble vitamins (FSV), carotenoids, and lipids including free-fatty acids (FFA) and triacylglycerols (TAG). NPLC of these compounds suffers long runtime, slow equilibration and poor reproducibility. Reversed Phase LC (RPLC) separation of these compounds is also possible but has potential sample carryover issue and requires more stringent sample clean-up to remove fat and other hydrophobic materials. Classes of compounds such as FFA are analysed by Gas Chromatography (GC) after derivatization into the methyl esters. Derivatization is time consuming and has a risk of re-arrangement of FFA. Also, for high carbon FA, their low volatility may cause inaccurate quantification.

METHODS AND RESULTS

Nine representative FSV and carotenoids have been successfully separated simultaneously by UPC² within four minutes on a single C_{18} column. These FSV and carotenoids include vitamin A acetate and palmitate, alpha-tecopherol and its acetate, vitamin D_2, vitamin K_1 and K_2 (MK4), beta-carotene and lycopene. The repeatability (n=6) of all the nine compounds was less than 0.25% in retention times (RT) and less than 2.6% in peak areas. RPLC separation of these compounds has potential sample carryover issue and requires more stringent sample clean-up to remove fat and other hydrophobic materials.

Individual saturated FFA standards containing even carbon number C8-C24 were prepared at 0.1 mg/mL in chloroform and injected onto the UPC²/Xevo G2 QToF system. The ACQUITY UPC² HSS C18 SB 1.8 µm (2.1 x 150 mm) column provides a reversed-phase like separation which results in effective resolution of the different FFA species. The gradient is run under acidic conditions using a small percentage of formic acid (0.1% v/v in methanol) to improve the peak shape and decrease peak tailing. The developed UPC²/Xevo G2 QToF method was applied with minor modifications for the profile of FFA in algae and algaenan extracts treated at low (310 ºC) and high (360 ºC) pyrolysis temperatures. The UPC² method does not require derivatization and is 10X faster (only 3 min run) than GC/MS and RP-LC methods and uses less toxic and cheaper CO_2 as a solvent.

TAGs in three common edible oils, specifically peanut, sunflower seed, and soybean oils, were separated on a UPC² C18 column using the UPC²/Xevo G2 QToF system with a gradient elution. All TAGs eluted within 15 minutes and showed baseline separation for all the major TAGs. This is much faster than the HT-CGC and the NARP-LC methods, which usually take 30-80 minutes. Compared to UPLC methods, UPC² has similar run time and resolution, but the column pressure in UPC² is much lower, which allows for higher flow rate or longer column to be used. The solvent consumption in UPC² is also lower.

CONCLUSION

Preliminary studies of using UPC² for the separation of fat-soluble vitamins (FSV), carotenoids and lipids are presented here to illustrate the performance of UPC² technology in these important analysis areas. UPC² provides fast, reliable, and simultaneous separation of multiple analytes in a single run while simplifying the sample preparation (no derivatization, direct injection of organic solvants), which indicate that UPC² is a promising chromatographic technique for the analyses of these classes of compounds.

REFERENCES

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