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Application Note

A Green and Comprehensive Approach for Palm Phytonutrients Analysis Using ACQUITY UltraPerformance Convergence Chromatography (UPC^2)

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Phytonutrients that are found in palm oil such as vitamin E, phytosterols, and squalene exhibit diversified physiological and pharmacological effects. Isolating these bioactive minor components has always been a major topic in the realm of analytical chemistry. However, separation of these palm phytonutrients using various liquid chromatography methods is challenging due to the long analysis time, the need for multiple methods, and usage of hazardous solvents during analysis. In this study, we developed a green alternative, more efficient separation method with Waters' ACQUITY UltraPerformance Convergence Chromatography (UPC^2) technology using supercritical CO_2 as mobile phase for the analysis of vitamin E, phytosterols, and squalene.

Benefits

A single direct method with minimal sample preparation was developed for the analysis of vitamin E, phytosterols, and squalene using Waters' ACQUITY UltraPerformance Convergence Chromatography (UPC^2) System coupled with a Photodiode Array (PDA) Detector

Introduction

Palm phytonutrients constitute ca. 1% of the total composition in palm oil, including carotenoids (500–700 ppm), vitamin E (600–1000 ppm), phytosterols (326–527 ppm), and squalene (200–500 ppm). Because of their exceptional biological activities and nutritional benefits, determining the composition of these minor constituents has then become a priority. Various HPLC methods have been developed to separate these phytonutrients (vitamin E, phytosterols, squalene) using HPLC with either normal phase (NP) or reverse phase (RP) chromatography. These conventional methods however, suffered from lengthy analysis time (up to 45–60 mins per analysis), tedious sample preparation or conversion between systems (NP to RP, or vice versa), as well as usage of toxic solvents such as MTBE for the separation.[1]

Convergence chromatography, a green technology separation technique using supercritical CO_2 offers an alternative approach to overcome the shortcomings of existing LC methods. With Waters' ACQUITY UPC^2 System, users will benefit from the orthogonal capability often associated with NP LC and the ease of use and method development simplicity often associated with RP LC. Waters' ACQUITY UPC^2 System uses supercritical CO_2 as weak solvent for the mobile phase which can be combined with various co-solvents in

the eluotropic series, enhancing the selectivity of the mobile phase and increasing the solvating power of the supercritical CO_2.

Results and Discussion

The main phytonutrients of interest from palm oil are vitamin E, squalene, and phytosterols, the structure of which are given in Figure 1. Waters Viridis HSS C_{18} SB Column (1.8 µm, 3.0 x 150 mm, p/n: 186006685 < https://www.waters.com/nextgen/us/en/shop/columns/186006685-viridis-hss-c18-sb-column-100a-18--m-3-mm-x-150-mm-1-pk.html>) was selected for the separation to provide a higher retentivity for these non-polar analytes. Pure palm phytonutrients of squalene, β-sitosterol, stigmasterol, compesterol, α-tocopherol, α-tocomonoenol, α, β, γ, and δ-tocotrienols were analyzed using the ACQUITY UPC^2 System coupled with an ACQUITY UPC^2 PDA Detector. Figure 2 shows the analysis of various phytonutrient standards using mobile phase compositions of supercritical fluid CO_2 and acetonitrile (as the organic modifier) at a flow rate of 1.0 mL/min and UV detection from 190 nm to 400 nm. A total of 10 palm phytonutrients were successfully determined in a single analysis compared to conventional HPLC methods where the determination of these phytonutrients has to be carried out by three different methods. We further tested the performance of the method with analysis of an unsaponifiable palm phytonutrient concentrate, a by-product in the process of palm-based biodiesel production from crude palm oil. These palm phytonutrient concentrates are rich in vitamin E, squalene, and phytosterols and they have received much attention in the palm oil industry due to their potential commercial value. Figure 3 shows the chromatogram from a palm phytonutrients concentrate where the 10 common phytonutrients present in palm oil were successfully separated and detected within a single injection. No lengthy or complicated sample preparation steps were required for this analysis. Palm phytonutrients concentrate samples were dissolved, filtered, and injected into the ACQUITY UPC^2 System. Separation of palm phytonutrients using Waters' ACQUITY UPC^2 technology, provides a more efficient, cost saving, and environmentally friendly approach using supercritical CO_2 as the mobile phase.

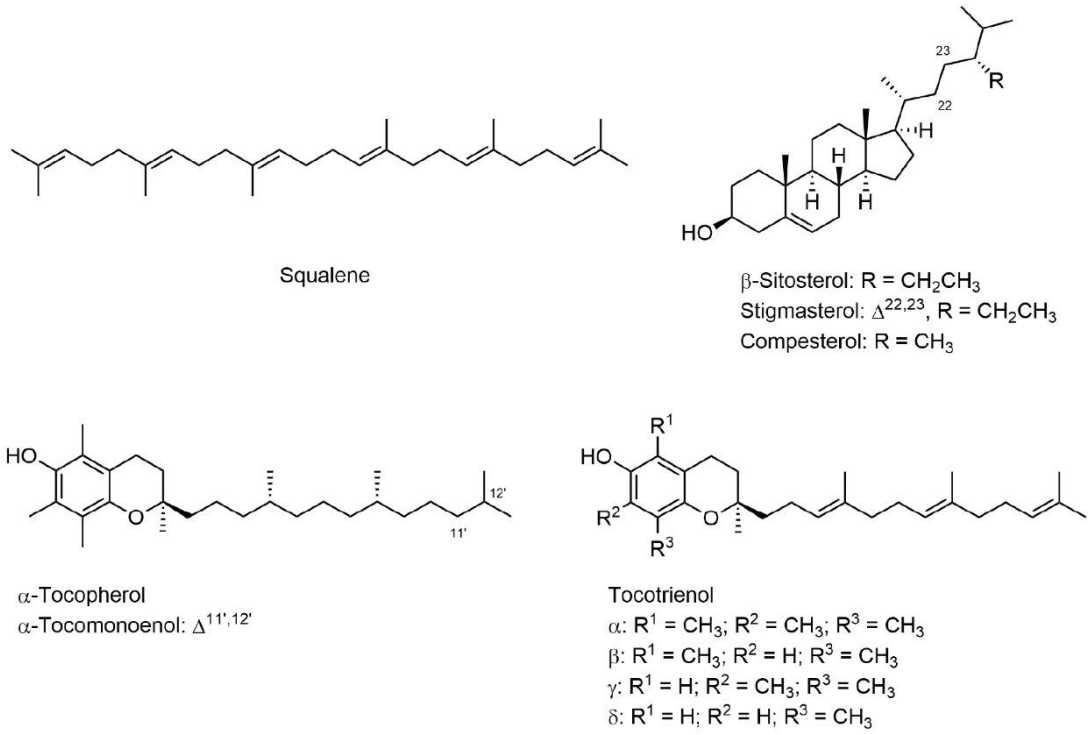

Figure 1. 10 common phytonutrients in palm oil.

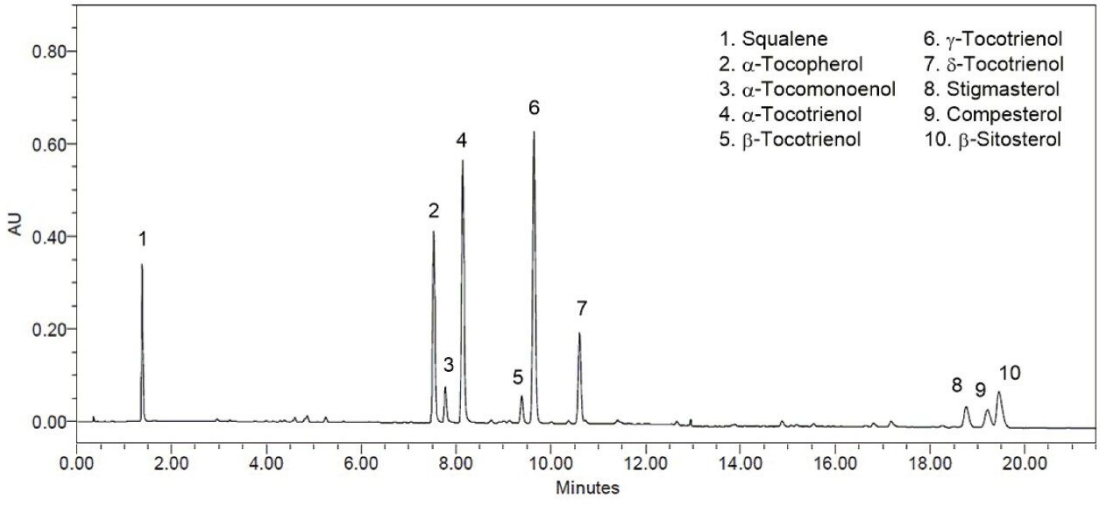

Figure 2. Max Plot chromatogram of the separation of palm phytonutrients using Viridis HSS C_{18} SB Column (1.8 µm, 3.0 x 150 mm) with a gradient separation of 1% ACN to 22% ACN as co-solvent in 20 minutes.

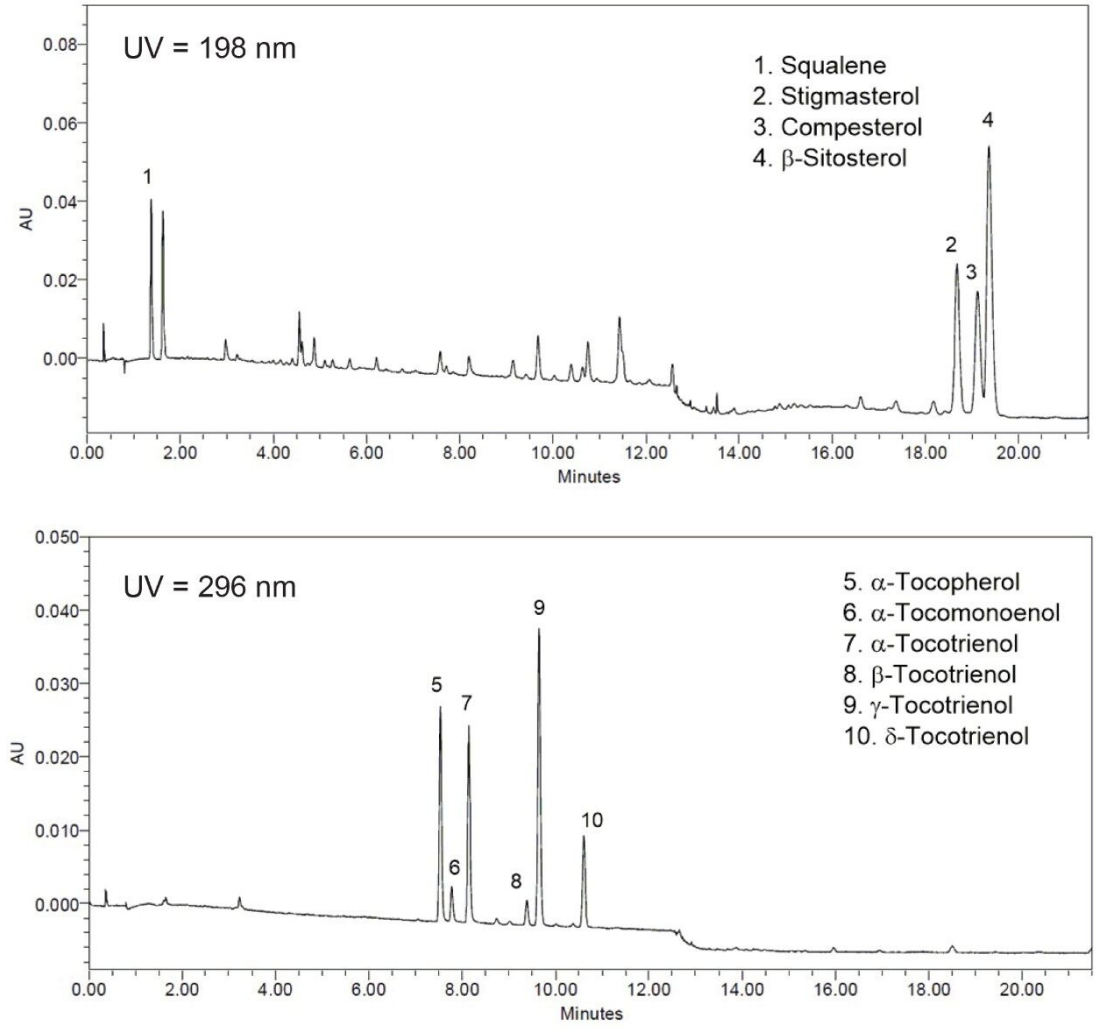

Figure 3. Chromatograms of the separation of palm phytonutrient concentrate extracted at UV wavelengths of 198 and 296 nm.

Conclusion

A total of 10 palm phytonutrients have been successfully separated and detected using Waters' ACQUITY UPC^2 System coupled with ACQUITY UPC^2 PDA Detector. This simple analysis method requires very minimal sample preparation and the phytonutrients can be determined in a single analysis, providing a more efficient, greener, and cost-effective method compared to any conventional HPLC methods.

References

1. Rathi D. N., Liew C. Y., Mohd Fairulnizal M.N., Isameyah D., Barknowitz G. Fat-Soluble Vitamin and Carotenoid Analysis in Cooking Oils by UltraPerformance Convergence Chromatography. *Food Anal. Methods* 2017 10, 1087–1096.

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