



UHPLC Method Development for Studying the Impact of Northern Growth Latitude and Environmental Factors on Tocopherols and Tocotrienols of Sea Buckthorn



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INTRODUCTION

Sea buckthorn is well known to contain several health-beneficial compounds, including tocopherols and tocotrienols¹. These compounds are traditionally analyzed with the high-pressure liquid chromatography (HPLC) method, but method development to ultra-high pressure liquid chromatography (UHPLC) could bring many advantages to the analysis.

UHPLC equipment reaches higher back pressures due to smaller particle sizes in the column, enabling faster analysis times and lower solvent consumption. Also, better analyte separation and more sensitive detection are possible.

MATERIALS AND METHODS

New UHPLC method was developed by trying different analysis conditions and comparing the results to find the best settings. Optimization was performed with a standard sample including all four tocopherols.

These parameters were tested:

- Flow rate with the scale of 0.1 - 0.6 ml/min
- Different injection volumes
- Different solvent composition ratios
- Increasing oven temperature

The reproducibility of the method was also tested.

PRELIMINARY RESULTS

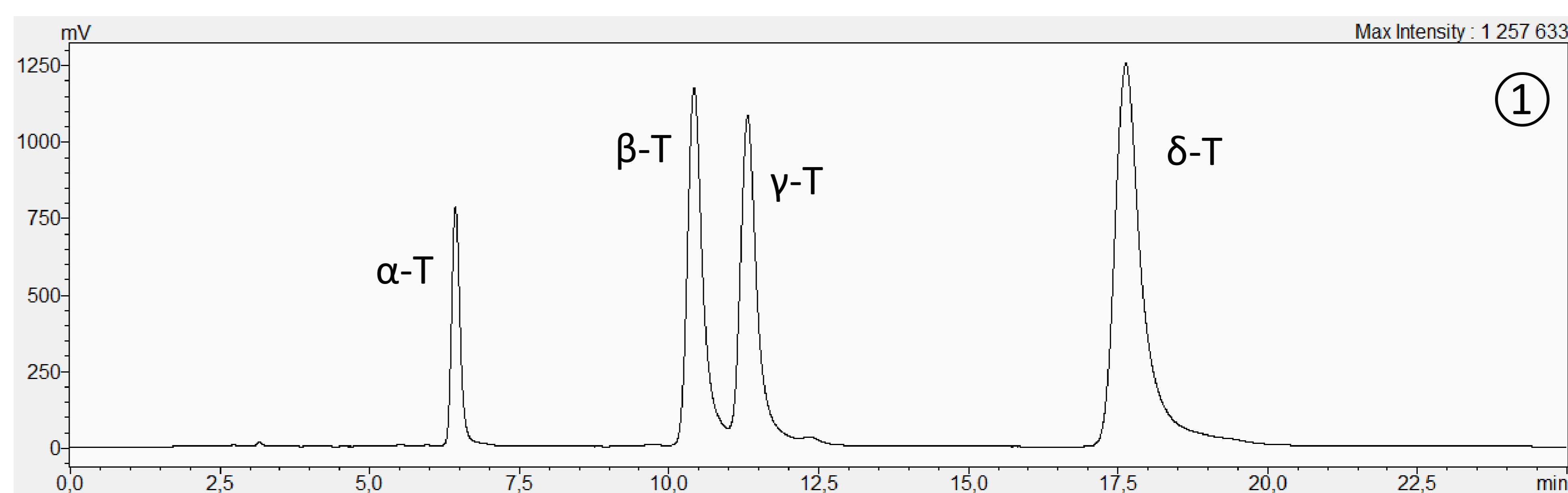
Method development enabled reduced analysis time to about ten minutes instead of the previous 25 minutes. Also, solvent consumption was able to reduce by 92 %. With the traditional HPLC method, solvent consumption was 2.0 ml/min, and with the new UHPLC method, consumption was able to decrease to 0.4 ml/min. Increasing the oven temperature did not add any value to the method.

AIM OF THE STUDY

In this study, the traditional HPLC method was upgraded to the UHPLC method. The aim was to reduce analysis time and decrease solvent consumption.

Also, analyte separation is desired to be at the same level as with the old method.

Chromatogram with HPLC



Chromatogram with UHPLC

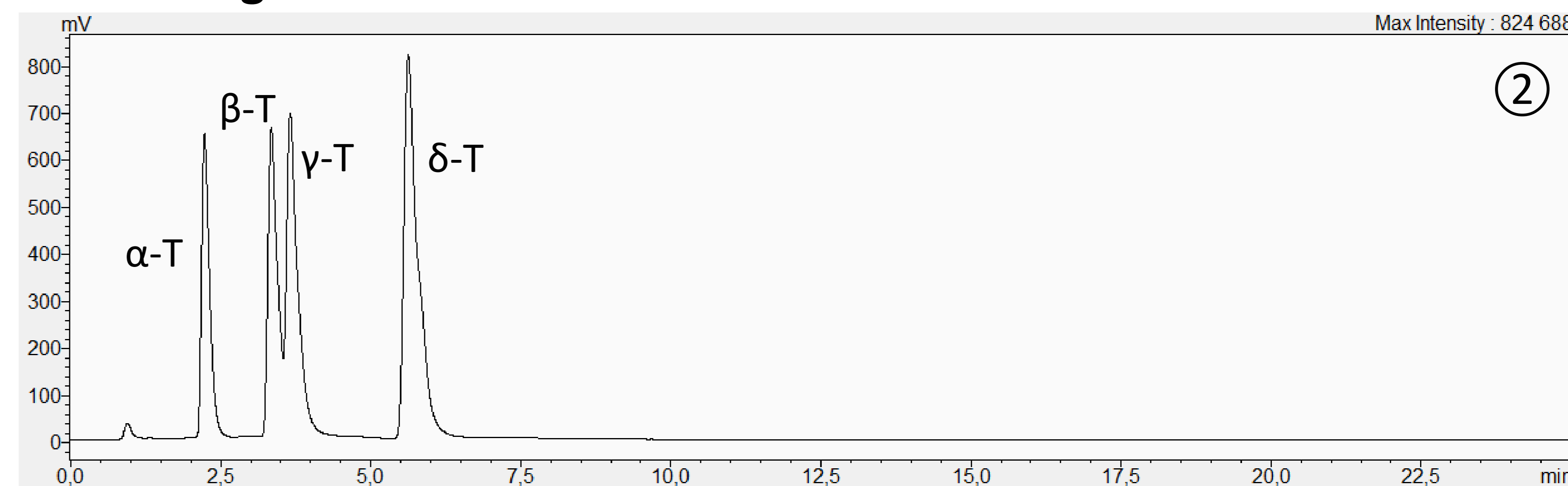


Figure 1 & 2. α -tocopherol (α -T), β -tocopherol (β -T), γ -tocopherol (γ -T) and δ -tocopherol (δ -T).

	① HPLC	② UHPLC
Flow rate	2.0 ml/min	0.4 ml/min
Analysis time	25 min	~ 10 min
Oven temperature	30 °C	30 °C
Solvent comp.	3 % 1,4-dioxane, 97 % heptane	2 % 1,4-dioxane, 98 % heptane
Column	Phenomex Luna 3 μ m Silica, 250 x 4.6 mm	Restek Pinnacle 1.9 μ m Silica, 100 x 2.1 mm

NEXT STEPS

The study is still ongoing, but when the method development is fully completed, the new method will be used to determine and compare the concentrations of tocopherols and tocotrienols in sea buckthorn berries. The intention is to discover how the berries are affected by different growth areas and environmental impacts.

References:

1. Zadernowski, R., Naczki, M. and Amarowicz, R. (2003) 'Tocopherols in Sea Buckthorn (*Hippophaë rhamnoides* L.) Berry Oil', *Journal of the American Oil Chemists' Society*, 80(1), pp. 55–58. doi:10.1007/s11746-003-0650-z.