Development of a Bi-dimensional HPLC method for the Stereospecific Analysis of Triacylglycerols

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A high performance liquid chromatography method with evaporative light scattering detection was developed for the analysis of stereospecific triacylglycerols (TAG), which allowed good separation of TAG species in 50 min, including regeneration of the column. The influence of the mobile phase, flow rate and column temperature on separation and analysis time were evaluated with positional isomers standards. An optimal separation was obtained with a heptane/acetone gradient at 25 °C and at 1 mL/min. The developed method was then used in a multidimensional determination of the TAG species and positional isomers in fats and oils blends. The bi-dimensional system was attained through the combination of non-aqueous reversed-phase (NARP) HPLC and silver ion (Ag)-HPLC. The primary column eluate was fractionated and the fractions were then injected into the secondary column, allowing the separation of TAG positional isomers that were not determined in the first dimension.