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Short communication

Analysis of triacylglycerols on porous graphitic carbon by high temperature liquid chromatography

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Abstract

The retention behaviour of several triacylglycerols (TAGs) and fats on Hypercarb®, a porous graphitic carbon column (PGC), was investigated in liquid chromatography (LC) under isocratic elution mode with an evaporative light scattering detector (ELSD). Mixtures of chloroform/isopropanol were selected as mobile phase for a suitable retention time to study the influence of temperature. The retention was different between PGC and non-aqueous reversed phase liquid chromatography (NARP-LC) on octadecyl phase. The retention of TAGs was investigated in the interval 30–70 °C. Retention was greatly affected by temperature: it decreases as the column temperature increases. Selectivity of TAGs was also slightly influenced by the temperature. Moreover, this chromatographic method is compatible with a mass spectrometer (MS) detector by using atmospheric pressure chemical ionisation (APCI): same fingerprints of cocoa butter and shea butter were obtained with LC–ELSD and LC-APCI–MS. These preliminary results showed that the PGC column could be suitable to separate quickly triacylglycerols in high temperature conditions coupled with ELSD or MS detector.

Keywords: Porous graphitic carbon; Triacylglycerols; Cocoa and shea butter; Column temperature; Liquid chromatography

1. Introduction

The European Commission has recently published a new directive [1] about chocolate specifying the maximum amount of cocoa alternatives so-called cocoa butter equivalents (CBE) [2]. These are mostly mixtures of various vegetable fats such as shea butter. This European directive requires the implementation of analytical methods to control its application and to discover cases of fraud. In order to comply with this regulation, a method has been proposed using reversed-phase liquid chromatography on octadecyl bonded stationary phase with evaporative light scattering detection (ELSD) [3]. As the composition of the mobile phase influences the droplet size and the detector response, isocratic mode gives a response which is practically independent of the solute in homologous series [4]; that's why this mode has been chosen in this study although gradient elution permits higher efficiency.

According to the conditions proposed by Dionisi et al. [3], the analysis of TAGs was long (about 54 min) although recent works have shown fast analysis of TAGs on ODS columns [5–7]. A simple method to decrease the analysis time consists in increasing the temperature of elution, but that is not suited for all bonded silica. Only few bonded silica can be used with temperature higher than 70 °C and used for lipid analysis [8,9].

We therefore have investigated the potentiality of porous graphitic carbon (PGC) which can be used at high temperature [10] This support possesses rigid and planar surfaces and affords two types of solute–adsorbent interactions: dispersive interactions and polar retention effect of graphite (PREG) [11,12]. It represents an underutilised stationary phase for RPLC of lipophilic compounds and has shown a potential for the discrimination of lipid species containing carbon double bonds [13,14] and glycolipids [15,16]. Interactions between the carbon double bond and the surface of PGC can be expected due to its polarisable nature, the number of double bonds and the conformation of the molecule. In the few studies with non-aqueous mobile phase with PGC [13,17,18], the increase in the hydrocarbon chain length of the molecule always induced an increase in retention whereas retention and selectivity between saturated and

Abbreviations: ELSD, evaporative light scattering detector; PGC, porous graphitic carbon; TAGs, triacylglycerols

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unsaturated fatty acid methyl esters (FAMEs) were compared in different mobile phases [19]. The objective of the present work was to design an efficient and rapid chromatographic method to separate TAGs on PGC with cocoa and shea butters as model samples. The effect of experimental parameters such as organic solvent ratio and column temperature, on the chromatographic behaviour of the TAGs, is discussed here.

2. Materials and methods

2.1. Chemicals

Chloroform, and isopropanol (HPLC gradient grade) were purchased from J.T. Baker (Noisy le sec, France). Tripalmitolein PoPoPo (16:1, *cis*-9), tripalmitelaidin PaPaPa (16:1, *trans*-9), tripalmitin PPP (16:0), trilinolein LLL (18:2, *cis*-9,12), trilinolenin LnLnLn (18:3, *cis*-9,12,15), tripetroselinin PePePe (18:1, *cis*-6), triolein OOO (18:1, *cis*-9), tristearin SSS (18:0) and trierucin EEE (22:1, *cis*-13) were obtained from Sigma (Saint Quentin Fallavier, France). Cocoa butter and shea butter were purchased at factories. All the samples were dissolved in chloroform at a concentration of 0.1 mg/mL.

2.2. Apparatus

The liquid chromatographic system consisted of a Jasco (Nantes, France) model PU-2085 pump, a Rheodyne (Berkeley, CA, USA) model 7725 injector with a 5 μL sample loop and an evaporative light scattering detection (ELSD) system (Sedere, Alfortville, France) model Sedex 85. The usual ELSD detector settings were as follows: drift tube temperature 45 °C, nebuliser gas pressure 3.5 bar, photomultiplier 8. The PGC separation was carried out on a Hypercarb® column (150 mm × 2.1 mm ID, particle size 5 μm) from Thermoquest, Hypersil (Runcorn, UK). The column was placed in a Jet Stream 2 oven (WO Industrial Electronics, Langenzersdorf, Austria). The flow rate was set at 0.2 mL/min. Data were collected and analysed using EZ-Chrom version 6.7 software.

LC–MS analyses were carried out using a Perkin-Elmer (Toronto, Canada) model LC-200 binary pump and a Sciex (Forster City, CA, USA) API 300 triple quadrupole mass spectrometer with an APCI source in the positive ion mode. Operating conditions were as follows: nebuliser gas flow rate 1.22 L/min, curtain gas flow rate 1.31 L/min, temperature = 400 °C, needle current = 3 μA, declustering potential = 34 V, focusing potential = 340 V, entrance potential = 10 V. Mass spectra were acquired by scanning the range *m/z* 800–1000 in order to obtain ions without fragmentation and to compare the chromatograms with the universal response of ELSD. Unit resolution was used as usual during spectra, the dwell-time was set at 2 ms and the pause-time was 5 ms. Injections were done by a Perkin-Elmer series 200 autosampler fitted with a 20 μL loop. All the results were acquired with the Analyst version 1.3.1 software (Sciex Applied Biosystems). PGC separation in LC–MS was carried out on a Hypercarb® column (150 mm × 4.6 mm ID, particle size 5 μm) from Thermoquest, Hypersil (Runcorn, UK) at a flow rate of 1 mL/min. Column temperature was regulated by a Jet Stream oven at 60 °C. Although APCI enables TAG ionisation, it was improved by adding ammonium acetate (10 mM) to isopropanol in mobile phase without modification of the retention and the peak shape.

3. Results and discussion

To assess the retention behaviour of TAGs on PGC, the high hydrophobicity of the packing required non-aqueous mixtures as mobile phase. Several TAGs were first tested as probes and mixtures of chloroform–isopropanol ($CHCl_3$–iPrOH) were chosen as the more suitable for elution of cocoa and shea butters and ELSD response.

3.1. Influence of the composition of mobile phase

An analysis of five standards (PPP, LLL, LnLnLn, PoPoPo and PaPaPa) was performed on PGC at 60 °C using various $CHCl_3$–iPrOH mobile phases (from 0.6 to 0.9 $CHCl_3$ volume fraction). As expected in NARP-LC, the retention of TAGs on a PGC column decreases as the $CHCl_3$ amount is increased (Fig. 1), and a good linearity is observed for the plot of log *k* *versus* the volume fraction of $CHCl_3$ with a correlation coefficient r^2 higher than 0.99. The slopes of variation are similar and the average value of the slope was about 3.97, greater than for FAMEs) (2.5–2.8) [13] showing a stronger interaction of TAGs on this packing. Moreover, it appeared that selectivity between these TAGs was weakly affected by the content of $CHCl_3$ in the mobile phase. Nevertheless, selectivity slowly increased as the $CHCl_3$ ratio decreases more particularly for the LnLnLn/PaPaPa and LLL/PaPaPa couples. For all the following analyses, the $CHCl_3$/iPrOH (80:20) mixture was selected as mobile phase for a suitable retention time to study the influence of temperature.

3.2. Influence of the nature of analytes

In reversed phase liquid chromatography on octadecyl silica (ODS), TAGs are separated according to the combined effect of the chain-lengths of fatty acid moieties plus their degree of unsaturation: the logarithmic retention factor *k* of TAGs increases

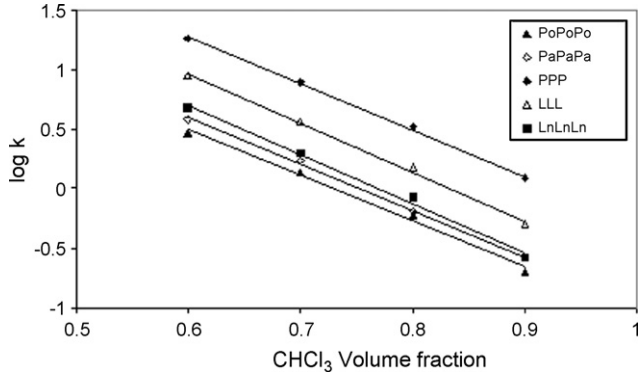

Fig. 1. Influence of $CHCl_3$ ratio on the retention of five TAGs. Column Hypercarb (150 mm × 2.1 mm I.D.). Mobile phase: $CHCl_3$–iPrOH mixtures. Flow-rate: 0.2 mL/min. Column temperature: 60 °C. ELSD.