Approaches to The Analysis of Saturated and Mono-Unsaturated FAME Using Highly Polar GC Phases

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Introduction

The resolution of complex fatty acid (FA) mixtures is becoming an increasingly important task in the analysis of fats and oils for determining nutritional or nutraceutical value, the detection of adulterants and anti-nutrients (e.g. trans acids) and for the isolation of novel compounds. The use of highly polar GC phases of the bis-cyanopropyl-polysiloxane type is common for such analyses. While these phases are particularly effective for resolving analytes on the basis of unsaturation and carbon chain length, they suffer some loss of resolving power for the methyl ester derivatives (FAME) with a low degree of unsaturation and are prone to overloading for saturates and mono-unsaturates.

This study describes the first part of our investigation into alternative strategies for FA analysis by GC and GCMS in which the analyte chemistry is modified to influence chromatographic behavior. We aim to develop a method that allows (1) the simple conversion from FAME to an alternative derivative, (2) improve or normalize the solubility of FA derivatives in very polar GC phases to prevent low temperature overloading, (3) allow changes in retention time on a "class" basis across the FA field to shift them away from other analytes occupying the same chromatographic space and (4) provide a common dominant retentive chemistry for biscyanopropyl type phases with the ultimate aim of improving the capacity for mono-enic isomer resolution.

Experimental

Standards of positional C18:1 isomers (prepared from individual compounds), linoleic methyl ester isomers and linolenic methyl ester isomers (Supelco, PA, USA) were used for this study. N-Benzylamide derivatives were formed from the methyl esters by adding 50 µL of the methyl ester solution into a screw capped autosampler vial, evaporating the solvent and then adding 50 µL of benzylamine (98 %, Sigma-Aldrich, USA) and 2-3 grain sized crystals of ammonium chloride. The vial was heated to 120 °C for 2 hours then cooled. Dichloromethane was added slowly with agitation. A white precipitate formed in the vial and allowed to separate from the solvent. The solvent was sampled directly into the GC injection port for split injection or diluted appropriately in dichloromethane for splitless injection. Alternatively, the dichloromethane layer was washed sequentially with 500 µL of 0.5 M hydrochloric acid solution, 500 µL of water and dried with anhydrous sodium sulphate prior to injection.

Gas Chromatography Mass Spectrometry was performed on a 6890GC-5973N MSD (Agilent Technologies, CA, USA) equipped with an ETP electron multiplier (SGE Analytical Science, VIC, Australia) and a BPX70 column (60 m x 0.25 mm i.d., 0.25 µm film thickness, SGE). Injections of 2 µL were splitless at a temperature of 280 °C. The purge flow was 50 mL/min with a nominal inlet pressure of 98 kPa. For methyl esters, the oven temperature was programmed from 120 °C (hold for 2 min) to 300 °C (hold for 3 min) at 2 °C/min. For the higher boiling N-benzylamides, the oven temperature was programmed from 40 °C (hold for 2 min) to 250 °C at 20 °C/min then to 300 °C (hold for 5 min) at 2 °C/min. The carrier gas was helium at a flow rate of 1.0 mL/min in constant flow mode. EI mass specta were collected over the range 50-550 Da at 2 scan/sec. The transfer line temperature was 280 °C, the quadrapole was 150 °C and the source was 230 °C. Chromatographic data was acquired and processed using ChemStation software (Version 100 D.02.00.275, Agilent Technologies).

Results and Discussion

The use of amide derivatives of FA is well established for structural elucidation but not for their influence on GC separation [1]. For this purpose, we have initially selected the fatty acid benzylamide derivative (FABA) because they are formed readily and in high yield from the corresponding methyl esters without the need for harsh reaction conditions or time consuming micro-reactions. The FABA provide an unhindered aromatic moiety that exhibits a dominant interaction with the highly polar GC phases. This effect can be used to normalize the solubility and retentive character of all FA. The derivatives also give characteristic mass spectral fragmentation that can be useful for selective SIM analysis and, in most cases, a relatively abundant molecular ion (Figure 1).

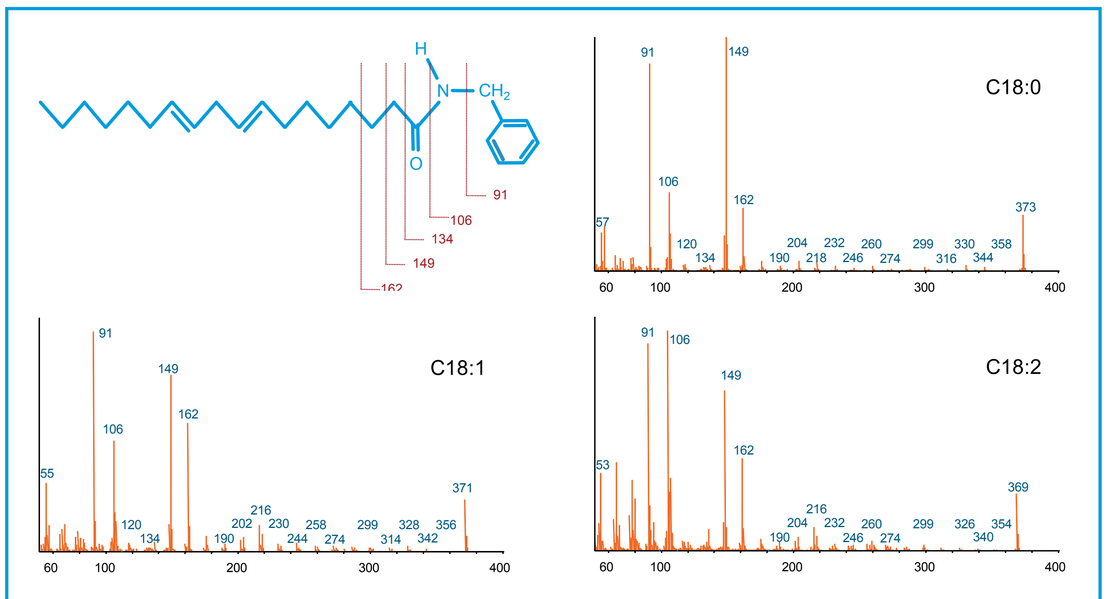

Figure 1: The EI mass spectra for N-benzylamide derivatives of C18:0, C18:1 and C18:2 fatty acids, and characteristic ions generated by the N-benzylamides (top).

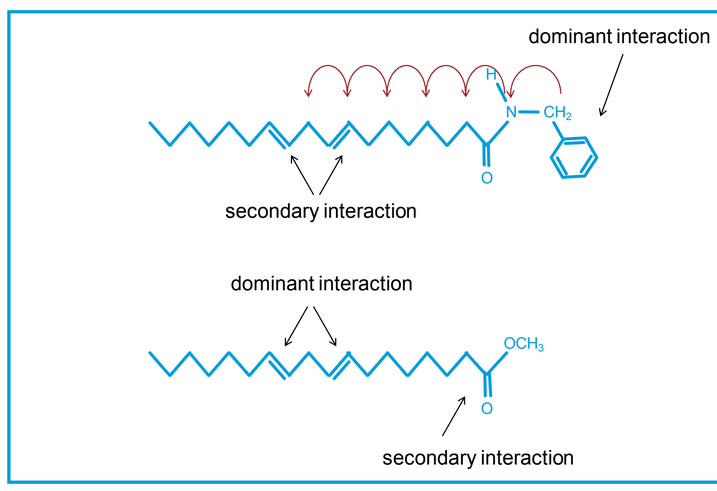

Figure 2: The use of an aromatic derivative gives a dominant retentive chemistry with biscyanopropyl phases and the structure dependant secondary retention may offer enhanced resolution over methyl ester derivatives. The amide derivatives have lower vapour pressures and therefore elute later than the analogous methyl esters.

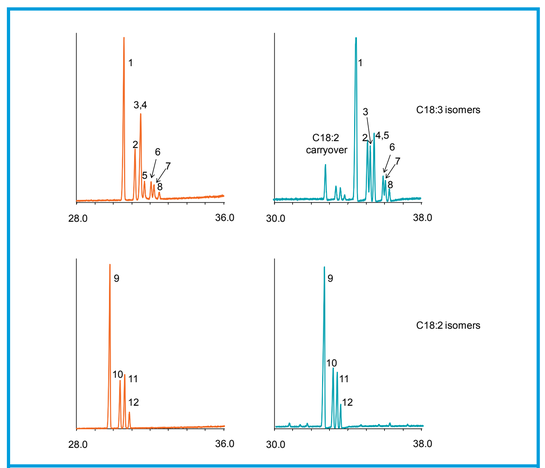

Figure 3: The separation of isomers of C18:3 and C18:2 FABA (left) and FAME (right). Octadecatrienoic acid isomers are 9t,12t,15t(1); 9t,12t,15c (2); 9t,12c,15t (3); 9c,12t,15t (4); 9c,12c,15t (5); 9c,12t,15c (6), 9t,12c,15c (7); 9c,12c,15c (8). Octadecadienoic acid isomers are 9t,12t (9); 9c,12t (10); 9t,12c (11); 9c,12c (12).

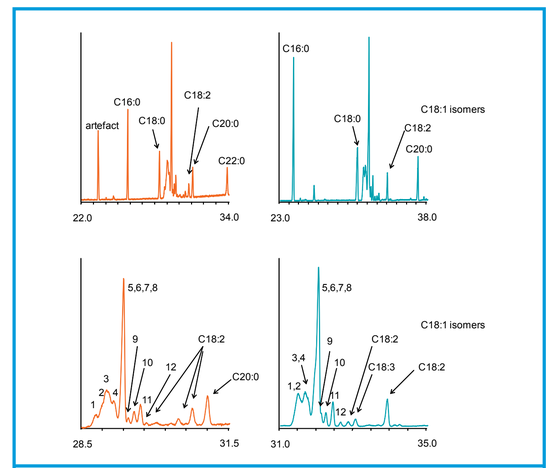

Figure 4: The separation of isomers of a complex mixture of C18:1 FABA (left) and FAME (right). Octadecenoic acid isomers are 12t (1); 11t (2); 9t (3); 7t (4); 12c (5); 6t (6); 11c (7); 5t (8); 9c (9); 7c (10); 6c (11); 5c (12).

Rational design of FABA predicted that a dominant π-π* retentive mechanism would operate for all derivatised FA and therefore improve solubility and selectively increase retention. With increased solubility and a common dominant point of retention, secondary retention through enic bonds was proposed that would be more sensitive to the position and geometric configuration of the double bond than would be observed for the corresponding FAME (Figure 2).

The separation of C18:2 and C18:3 FAME and FABA is shown in Figure 3. No significant difference in elution order or separation is observed for the C18:2 isomers studied. Some selectivity differences are noted for C18:3 isomers, particularly with respect to the elution of 9c,12t,15t-C18:3 relative to 9t,12c,15t- and 9c,12c,15t-isomers.

The separation of a complex mixture of C18:1 positional isomers is shown in Figure 4. Altered selectivity is apparent with resolution of 9c-C18:1 from the main group of 12c, 6t, 11c and 5t isomers and with increased separation occurring in the trans band. Also apparent in the C18:1 chromatogram is the spread of unsaturated C18 FABA between C18:0 and C20:0. This effect is demonstrative of the improved contact with the phase that results from the FABA derivative, despite the use of higher temperatures for elution. By maintaining intimate contact with the phase at the amide moiety, the secondary retentive interactions at the enic bonds are also able to exist for a longer period. Such an amplification technique is useful in the design of separation strategies.

The most significant changes to separation have been observed among the trans-isomers of FA rather than between cis and trans isomers. This preliminary observation suggests that the less hindered (and more linear) trans-isomers are more ameniable to the dual retention site mechanism and therefore that the mechanism is sterically controlled.

Conclusion

We have altered the separation of derivatised FA on a polar GC phase by incorporating a common functional group that is capable of giving a dominant π-π* retentive chemistry. The technique increases the relative retention of unsaturated FA relative to saturated FA, allows the separation of FA from coeluting peaks and shows increased selectivity on the basis of bond position, particularly for trans-FA.

The technique is intended to be most useful for the resolution of mono-enes by introducing a common dominant point of retention that then allows for selectivity differences based on the relative spacing and steric aspects of secondary retention about the enic bond. It is likely that the selectivity will be further enhanced by using more volatile derivatives that allow for movement of analytes through the column with less intramolecular energy. Further work using aromatic derivatives that elute at lower temperatures (including phenyl and benzyl esters) is continuing.

[1] Christie WW, Lipid Analysis - third edition. The Oily Press, Bridgwater (2003), www.lipidlibrary.co.uk.