

Technical Report

2-Dimensional Separation of Triacylglycerols in *Borago Officinalis* Using the Shimadzu 2D-LC System Coupled to an Evaporative Light Scattering and IT-TOF Detection

LCxLC for triacylglycerol analysis in borage

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Abstract:

Triacylglycerols (TAGs) are the major components of fats and oils from animal and vegetable sources. The high number of fatty acids combinations in the glycerol backbone can create an intricate pattern of compounds, difficult to separate under monodimensional HPLC analysis. This Application Note demonstrates the potential of comprehensive two-dimensional liquid chromatography (LCxLC) using the Shimadzu 2D-LC system with both an evaporative light scattering and ion trap-time of flight mass spectrometer (IT-TOF) detectors for the analysis of triacylglycerols in a complex sample.

Keywords: LCxLC, LCMS-IT-TOF, ELSD, triacylglycerols, *borago officinalis* oil

1. Introduction

The most applied methods for TAGs analysis are non-aqueous reversed-phase liquid chromatography (NARP-HPLC) and cation-exchange stationary phase loaded with silver ions (Ag⁺). In the RP-HPLC system, TAGs separation occurs according to the partition number (PN = CN – 2NDB; CN = carbon number, NDB = number of double bonds) and retention increases depending on the PN. On the other hand, the Ag⁺-HPLC technique provides separation on the basis of the degree of unsaturation of the TAGs, due to specific interactions between the double bonds and the silver ions bonded to the stationary phase. Due to the complexity of TAGs in oils and fats, neither Ag-LC nor NARP-HPLC are capable to provide reliable separation and identification of all TAG species. Consequently, multidimensional (MD) techniques have been increasingly used to unravel the complexity of TAGs in oils and fats.

In this technical report, we investigated a comprehensive two-dimensional LC system, comprised of a cation exchange micro-column loaded with silver ions (150 mmL. × 1.0 mmL.D.; 5.0 μm) in the first dimension (D1) and a partially porous column (50 mmL. × 4.6 mmL.D.; 2.7 μm) in the second dimension (D2), in combination with an evaporative light scattering detector (ELSD) and IT-TOF mass spectrometer, for the analysis of TAG fraction of a borage oil sample.

2. Experimental

2-1. Materials and Methods

All solvents were HPLC grade from Sigma-Aldrich. All standard TAGs (LaLaLa, LaLaM, LaMM, MMM, POP, PPO, OOO) were from Sigma-Aldrich; POS, SOO and SSO that were from Extrasynthese.

The borage oil was purchased at a local shop.

2-2. LCxLC Instrumentation

- Shimadzu CBM-20A controller
- two Shimadzu LC-30AD dual-plunger parallel-flow pumps
- two Shimadzu LC-20AD dual-plunger parallel-flow pumps
- Shimadzu DGU-20As degassing unit
- Shimadzu CTO-20A column oven
- Shimadzu SIL-30AC autosampler

For connecting the two dimensions: two electronically-controlled 2-position, 6-port high pressure switching valves FCV-32AH (with two 11 μL empty loops)

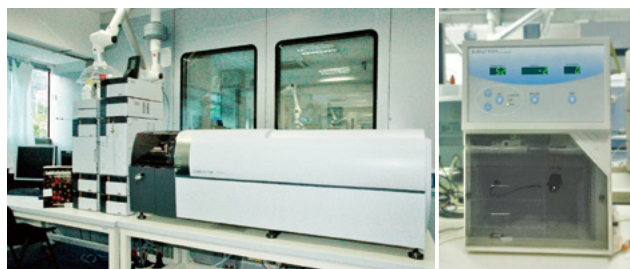


Fig. 1 LCxLC instrumentation

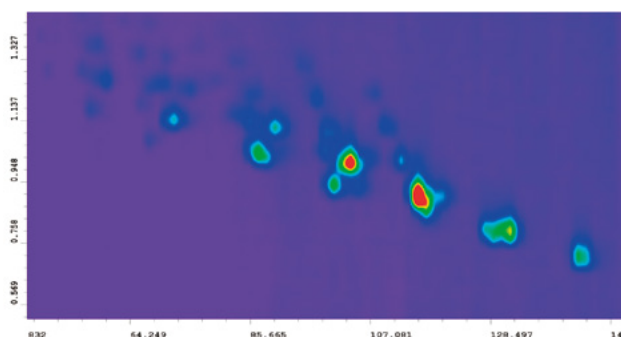


Fig. 2 LCxLC-ELSD plot of a borage oil sample

2-3. LC×LC-MS Conditions

1D separations: Ag custom column (150 mmL. × 1.0 mmI.D., 5.0 μm d.p.)

Flow rate : 7 μL/min
Mobile phases : (A) 1.5 % v/v of BCN in n-hexane and
(B) 2.4 % v/v of BCN in n-hexane
Gradient mode : 0 min 0% B; 40 min, 100% B, 150 min, 100% B.

2D separations: Ascentis Express C18 column
(Supelco, Bellefonte, PA, USA, 50 mmL. × 4.6 mmI.D., 2.7 μm d.p.)

Flow rate : 4 mL/min
Mobile phases : (A) ACN (B) IPA
Gradient mode : 0.01 min, 30% B; 0.08 min, 30% B; 0.10 min, 40% B;
1.20 min, 70% B; 1.21 min, 30% B; 1.51 min 30% B.
Modulation time of the switching valve : 1.5 min

2-4. Detector

Shimadzu ELSD: evaporative temperature: 58°C; nebulizing gas (N₂) pressure: 260 kPa; detector gain: <1 mV; sampling frequency: 2 Hz.

Shimadzu LCMS-IT-TOF (APCI source).

2-5. Software

- Shimadzu LCMSsolution software (Ver. 3.50.346)
- ChromSquare (Version 2.0) from Chromaleont, Messina, Italy

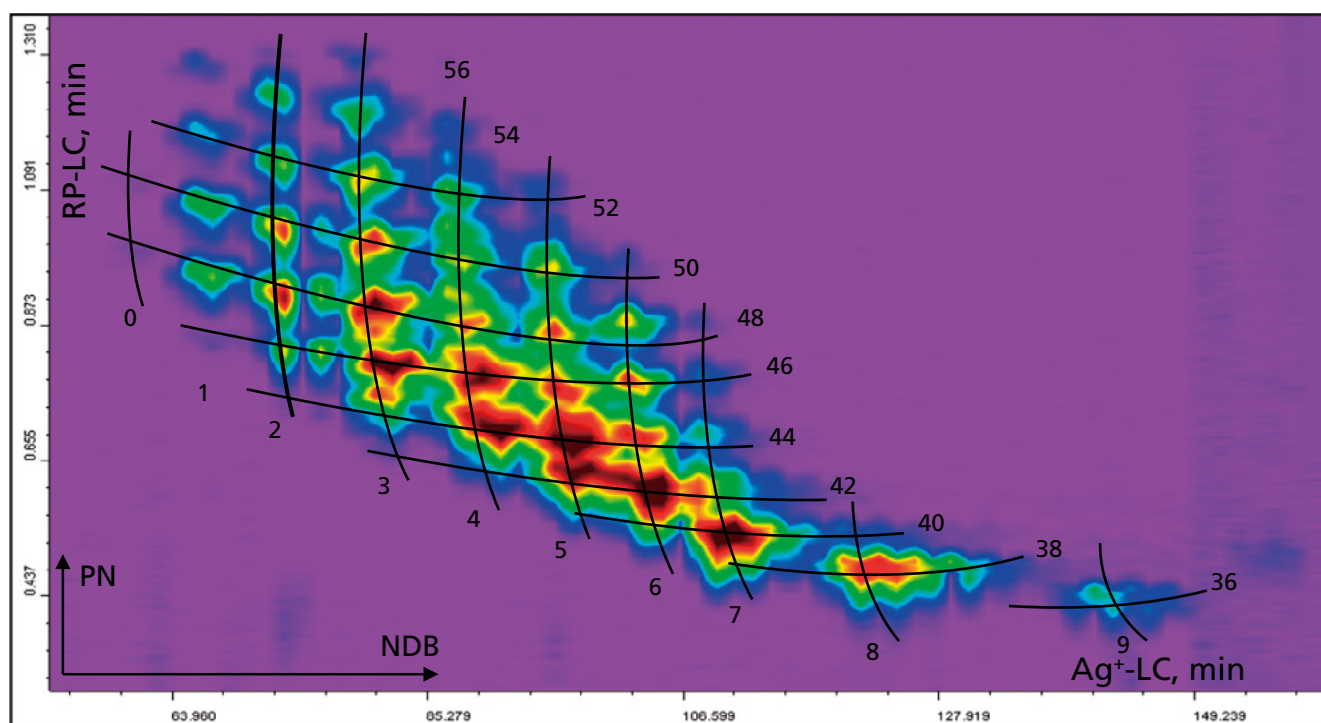


Fig. 3 Ag⁺-LC × RP-LC/APCI-MS of a Borago oil (*Borago Officinalis*) sample

3. Results and Discussion

Fig. 3 shows the contour plot of a borago oil sample. TAGs are separated in the D1, according to NDB, and in the D2, according to their PN. As can be appreciated, numerous different TAGs have been located in the 2D-LC retention plane through the inspection of the group-type patterns. TAGs found in the borago oil sample fell in the range of 1 and 9 NDB and PN values from 36 to 56.

TAGs with the same NDB but with different PN were easily resolved through the complementary separation mechanism shown by the two columns e.g. C24:1LyLn, C22:1LyLn, GLyLn, which co-elute under Ag-HPLC conditions, since they present the same degree of unsaturation, are well separated in RP-LC, due to their different PN number.

Moreover, also TAGs with the same NDB and with the same PN can be partially separated, as a consequence of the Ag-separation mechanism which enables the separation not only according to NDB but also to the distribution of the NDB within the FA chains in the TAG molecule.

4. Conclusions

The 2D set-up, thanks to the complementary separation selectivity provided by the two columns, allowed to locate a total of 89 TAGs in the 2D-LC retention plane making the approach useful for identification purposes. The use of the ChromSquare software allowed the visualization of the LCxLC data as a 2D plot (Fig. 3), 3D image (Fig. 4), the integration of the blob area for quantitative data analysis (fig. 5), the visualization of selected part of the plot, with the raw data analysis relative to one modulation (showing a different colour for every peak) (Fig. 6).

The coupling with the IT-TOF mass spectrometer added a third dimension, useful in the identification of every single component. The combination of the chromatographic information (position of the blob in the 2D plot, and the MS spectrum permits a reliable identification of every single component. See for example Fig. 7, where MS spectra of TAGs with increasing NDB are shown.

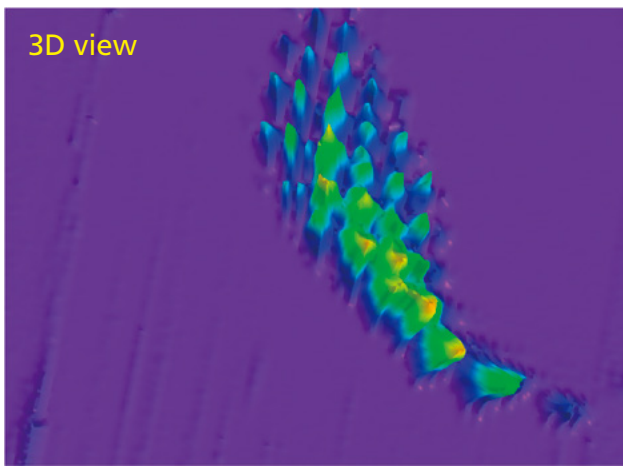


Fig. 4 3D view of the LCxLC analysis

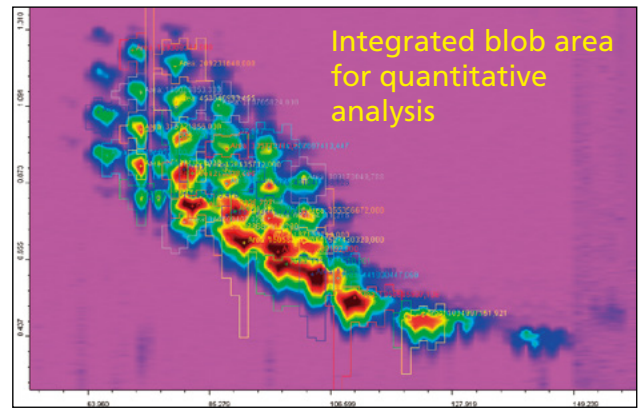
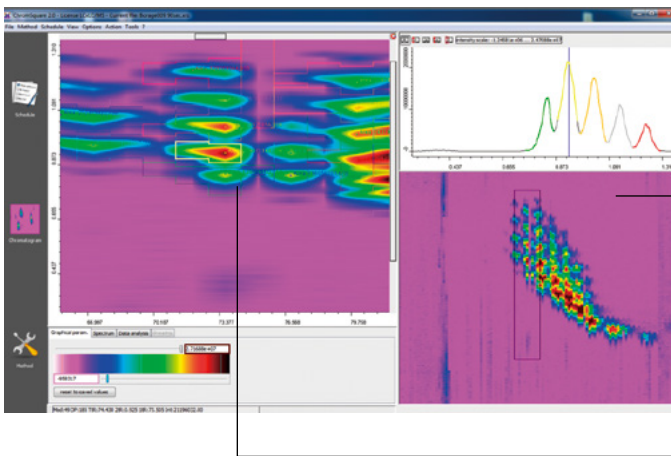


Fig. 5 Integrated blob area for quantitative data analysis



Raw data analysis showing 1 modulation time

Uninterpolated data

Expansion of the retention time window for 1 to 3 DB TAGs

Fig. 6 Raw data analysis window shows single modulation times, i.e. the number of “cuts” of the first dimension eluent. Different colours represent different components.

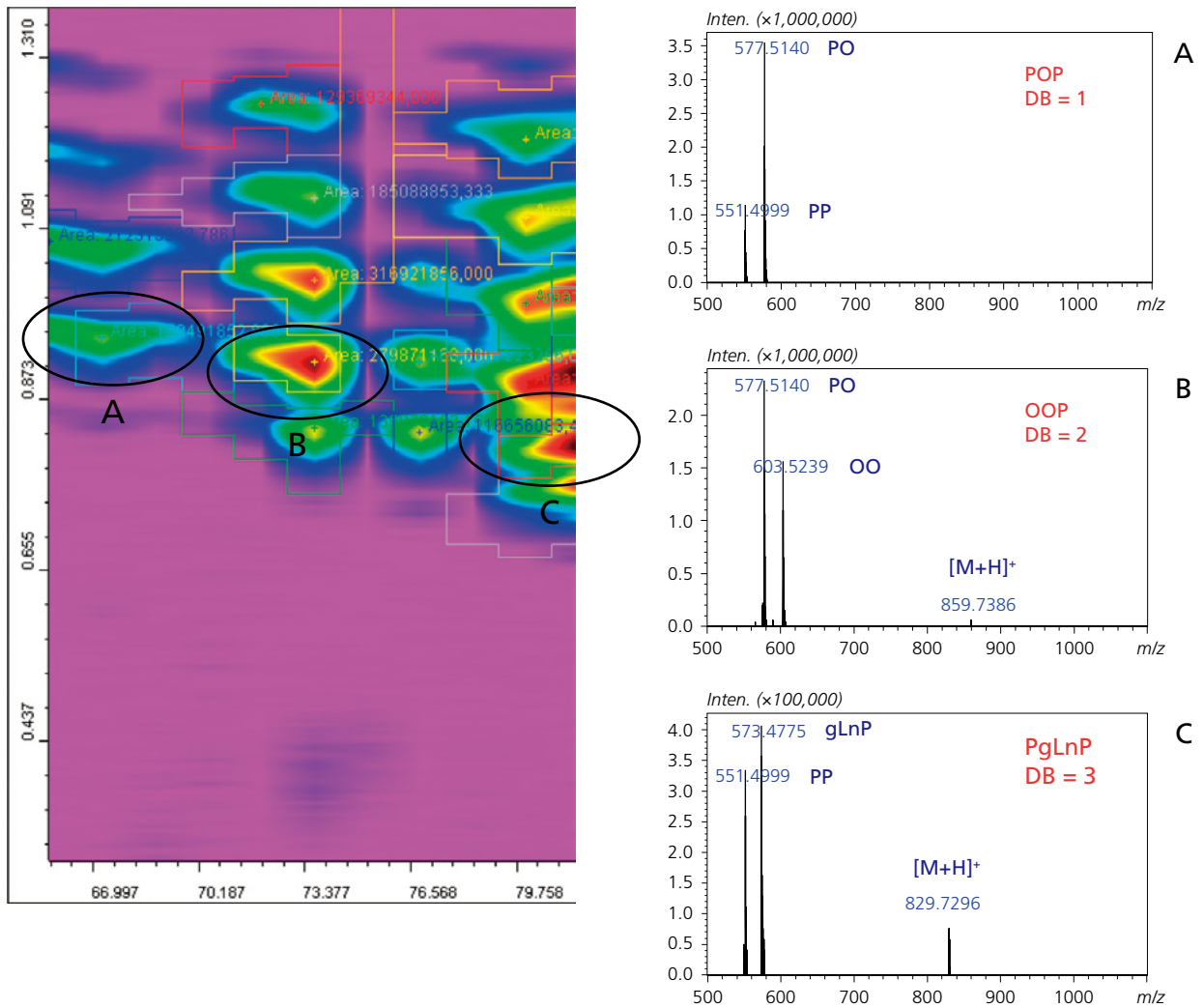


Fig. 7 Expansion of the LCxLC plot, and MS spectra of selected TAGs with a different DB number.