# Comprehensive LCxGC-TOF MS:a Novel Tool for (trans) Fatty Acid Analysis

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## INTRODUCTION

Fats and oil are an indispensable part of the human diet. The tri-acylglycerides in the oil/fat deliver energy as well as the fatty acids essential for the human body. A wide variety of fatty acids exists, differing in chain length or number, position and orientation (*cis/trans*) of the double bonds. Detailed analysis is needed to understand the effects of specific isomers on human health including carcinogenesis, artherosclerosis, platelet aggregation and body fat deposition.

Two chromatographic methods have been widely used in fatty acid analysis:

- Capillary gas chromatography (GC) with flame ionisation or mass spectrometric (MS) detection on highly polar columns, and
- High-performance liquid chromatography on silver-ion columns ($Ag^+$-HPLC).

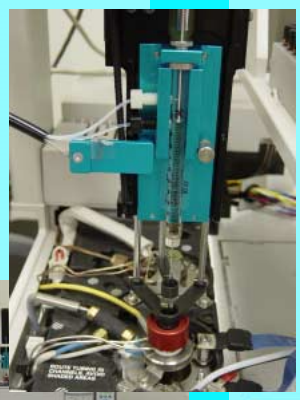
The most advanced method available today is combination of GC with $Ag^+$-HPLC. Two possible combinations have been described in literature:

- Serial combination of the methods where GC provides the total isomer content and $Ag^+$-HPLC the isomeric distribution, or
- Parallel combination where $Ag^+$-HPLC provides a pre-separation prior to a detailed GC/GC-MS analysis of a particular fraction.

In the present work a third option for coupling $Ag^+$-HPLC with capillary GC for fatty acid analysis is presented: Comprehensively coupled $Ag^+$-HPLCxGC–MS. In comprehensive two-dimensional chromatography each peak eluting from the first dimensional column is transferred to a second column for a second separation on a column with a different selectivity.

## INSTRUMENTATION

- Focus LCxGC interface (ATAS GL)
- Optic 3 injector (ATAS GL)
- 6890 GC (Agilent)
- Pegasus III ToF MS (LECO)
- Capillary GC column, VF-23ms, 30 m x 0.25 mm x 0.25 µm (Varian)
- Alliance 2690 HPLC system (Waters)
- HPLC column, $Ag^+$-phase, 25 cm x 4.6 mm x 5 µm (Varian)



## ANALYTICAL SYSTEM

**Figure 1:**
LCxGC –ToF MS system. $Ag^+$-phase column for LC separation (left insert), Syringe with side entrance used as interface between LC and GC (right insert).

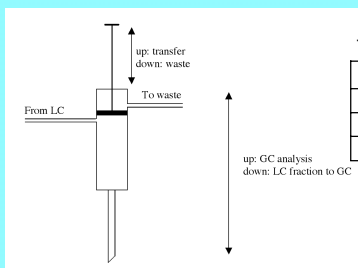



Table I: *Interface positions*

| | Needle | Plunger |
|---|---|---|
| **LC fraction to waste** | up | down |
| **LC fraction to GC** | down | up |
| **GC analysis** | up | up |

**Figure 2:** Comprehensive LCxGC interface. Schematic drawing.

**Figure 3:** Molecular structure of trans oleic acid methyl ester

## ANALYSIS METHOD

*TAG methylation*

- Dissolve 50 mg fat in 5 ml 5% $H_2SO_4$/MeOH
- Heat solution at 70°C overnight
- Add 5 ml cold HPLC grade water
- Extract FAMEs with 5 ml hexane
  (Note: Possibility of sample degradation!)

*LC∞GC analysis*

- Inject 20 µl on the $Ag^+$ ion exchange column
- The LC effluent is transferred into the Focus syringe through the side entrance of the syringe (See figure 1)
- Send first 2.5 minutes to waste
- When the GC system is ready, the syringe is inserted into the injector. Next, the LC pump is (re-)started to transfer a 250 µl fraction into the injector. After injection the pump is stopped, the syringe withdrawn from the injector and the GC analysis is started.

*Data processing*

- After completion of the entire LCxGC analysis, the GC–ToF MS data of all LC fractions is processed and plots are constructed.

## GENERAL SURFACE PLOT

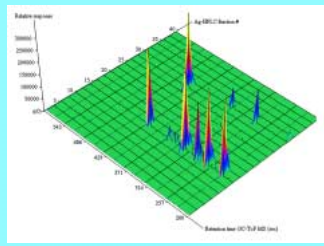


**Figure 4:** Surface plot of $Ag^+$-LCxGC analysis of butter FAMEs. Injection volume: 20µl, Fraction size: 250 µl, LC flow: 1 ml/min, LC eluent 100% dichloromethane, GC oven: 40°C / 50°C/min / 176°C(7.28 min), Column flow: 1 ml/min, ToF MS: mass range 50-500, 10 specs/sec.

## LC∞GC COMPARED TO LC AND GC

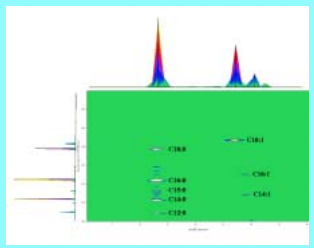


**Figure 5:** Colour plot of $Ag^+$-LCxGC analysis of butter FAMEs. Re-constructed GC–ToF MS chromatogram (*y*-axis projection) and re-constructed $Ag^+$-HPLC chromatogram (*x*-axis projection). Conditions see figure 4.

## SATURATED FAMEs

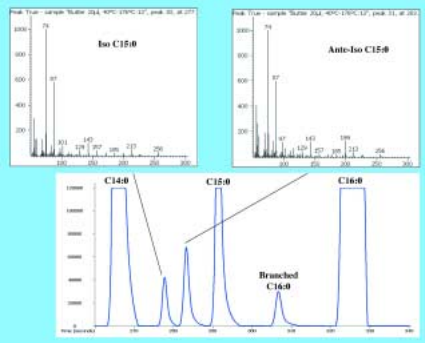



**Figure 6:** Part of GC–ToF MS chromatogram of $Ag^+$-LC fraction 13 (bottom). Small peaks between C14:0 and C15:0 are identified by the respective spectra as Iso C15:0 (upper left) and Ante-iso C15:0 (upper right).

## DEGRADATION DURING TRANS ESTERIFICATION

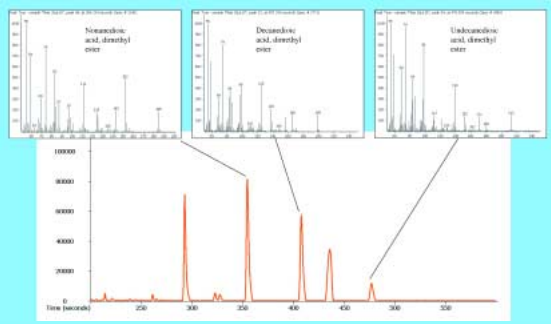


**Figure 7:** Fraction 18 of Corn FAMEs. Elucidation of degradation products by time-of-flight mass spectrometry.

## LC ELUTION BEHAVIOUR: INFLUENCE OF CHAIN LENGTH

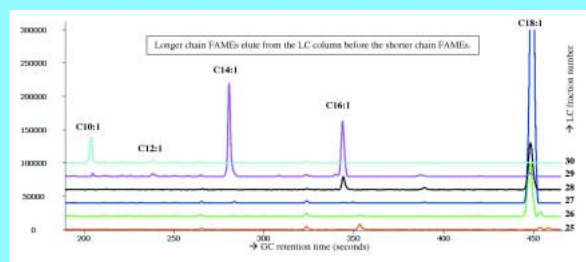



**Figure 8:** Part of GC–ToF MS chromatogram of $Ag^+$-HPLC fraction 25-30.

## ENHANCED RESOLUTION OF LC∞GC

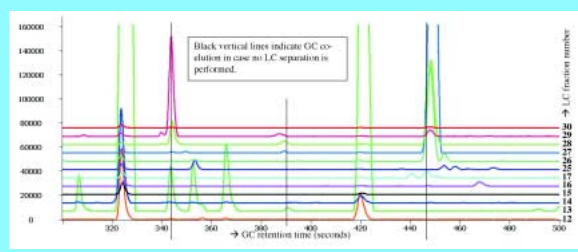



**Figure 9:** Overlay of fraction 12 – 17 + 25 – 30.

## CONCLUSIONS

- $Ag^+$HPLC and GC are complementary techniques in fatty acid analysis.
- LCxGC provides information that can not be obtained by LC or GC separately.
- Automated on-line LCxGC is possible using the ATAS Focus LCxGC interface.
- Sensitive mass spectrometry with automated deconvolution and identification is required to identify the many small peaks separated in LCxGC of fatty acids.