

The Use of Low-Pressure Positive Chemical Ionization GC/MS for the Characterization of Fatty Acid Methyl Esters (FAME)

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Overview

Purpose: Create a low-cost, reliable procedure for using low-pressure liquid chemical ionization with single quadrupole or ion trap GC-MS systems. The technique should be helpful in characterizing fatty acid methyl esters.

Methods: A reservoir of acetonitrile is attached to a length of fused silica that enters the MS through the transfer line of the gas chromatograph. Acetonitrile flow is regulated through a needle valve.

Results: Spectra were successfully generated in electron impact and low-pressure chemical ionization modes with both single quadrupole and external ionization ion trap MS. Standards and plant extracts showed fatty acid methyl ester spectra comparable to data reported in the literature. A greater amount of adduct [M+54] and [M+41] formation was seen in spectra generated from the ion trap, compared to the single quadrupole MS.

Introduction

Identification and quantification of polyunsaturated fatty acid methyl esters (FAME) by electron ionization (EI) using GC-MS is difficult due to the similar fragmentation patterns and loss of the molecular ion in many cases. Low-pressure positive chemical ionization (LP-PCI) that uses liquid acetonitrile to produce reagent ions provides a soft ionization process which produces an abundant molecular ion for individual FAME species. In addition, LP-PCI with acetonitrile provides greater sensitivity and longer time between MS source cleaning than with some other ionization techniques. The goal of this study was to create a simple, low-cost apparatus for running LP-PCI on two types of benchtop GC-MS systems for generation of reliable spectra similar to what has been previously reported on ion traps using factory installed LP-PCI options.

Methods

Thermo Scientific ITQ 1100 and ITQ 900TM external ionization ion traps and a Thermo Scientific DSQII single quadrupole MS were used. Each MS was connected to a Thermo Scientific TRACE GC Ultra equipped with a Programmable Temperature Vaporizing (PTV) inlet for sample introduction (Fig. 1). A sealed HPLC column was emptied of its packing and was used as the acetonitrile reservoir for LP-PCI. A length of fused silica was placed from the reservoir to a needle valve for flow control (Fig. 2). Another length of fused silica was positioned from the needle valve through the GC transfer line and into the source of the MS. A two-holed vespel/graphite ferrule allowed for both the LP-PCI fused silica line and the capillary column to span the GC heated transfer line (Fig. 3). Acetonitrile flow was regulated by the needle valve until an optimal production of *m/z* 40 (100%, base peak), 42 (30% of base peak) and 54 (30% of base peak) were obtained in the tune program. The needle valve was closed and acetonitrile was allowed to clear from the MS ion source for a few minutes prior to running samples in EI mode. Parameters for the GC-MS are shown in Table 1. One microliter of FAME standard or seed extract sample was injected into the GC either manually or via autosampler. Sample data were analyzed using Thermo Scientific Xcalibur ver. 2.0 software.

FIGURE 1. Basic setup of the GC-MS system used

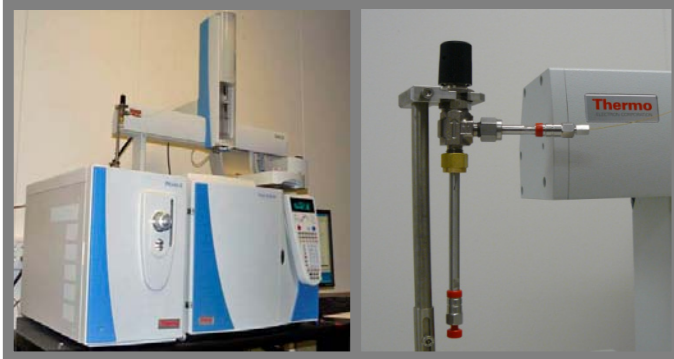


FIGURE 2. LP-PCI apparatus showing the acetonitrile reservoir and needle valve

TABLE 1. Selected instrument parameters used in the LP-PCI and EI analyses

Capillary Column	ITQ 1100 MS Parameters (LP-PCI)
Restek Rxi [®] -5MS, 30 m x 0.25 mm i.d. x 0.25 µm film thickness	Source temp 220 °C, start time 3.0 min Damping gas flow at 0.3 ml/min Microscans 2
TRACE GC Ultra [™] Oven Parameters	DSQII [™] MS Parameters (EI)
Initial temp 50 °C, hold 1.00 min, ramp at 10 °C/min to 300 °C, hold 5.00 min Carrier gas - helium Constant flow 2.0 ml/min PTV splitless injection mode	Source temp 230 °C, start time 3.0 min Tune file: autotune Full scan 55-550 amu Electron lens 10 V Electron energy 70 eV Emission current 200 µamp
PTV Inlet Parameters	DSQII [™] MS Parameters (LP-PCI)
Initial temp 80 °C, inject 0.1 min, transfer rate 14.5 °C/sec to 280 °C, hold 5.0 minutes	Source temp 220 °C, start time 3.0 min Tune file: manual tune on <i>m/z</i> 40, 42, 54 Full scan 100-550 amu Electron lens 10 V Electron energy 120 eV Emission current 200 µamp
ITQ 1100 [™] MS Parameters (EI)	
Source temp 230 °C, start time 3.0 min Damping gas flow at 0.3 ml/min Microscans 2 Maximum ion time 25 ms Tune file: autotune Full scan 55-550 amu Electron lens 10 V Electron energy 70 eV Emission current 200 µamp	

Results

ITQ 1100 Ion Trap – FAME Standard Samples

Spectra of saturated FAME standards run in EI and LP-PCI using acetonitrile showed more fragmentation in EI mode, but a large [M+H]⁺ fragment in LP-PCI mode. With LP-PCI, saturated FAME species showed a 40 amu adduct, typical of spectra reported previously¹ (Fig. 4). In contrast to saturated FAME, the acetonitrile LP-PCI spectrum of unsaturated FAME usually showed the presence of four characteristic ions in addition to the 40 amu adduct: MH⁺, [MH-32]⁺, [MH-32-18]⁺ and [M+54]⁺.^{2,3} A good example of these ions was seen with a C:22:6n3 cis 4, 7, 10, 13, 16, 19 FAME standard obtained on the ion trap with LP-PCI (Fig. 5).

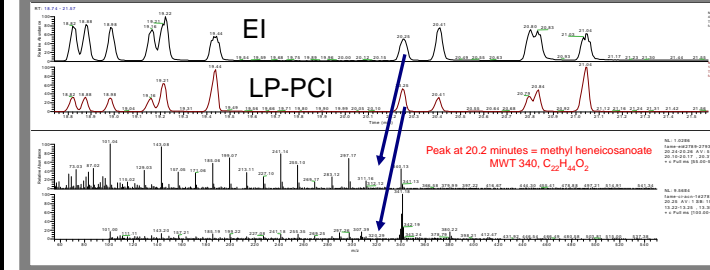
DSQII Single Quadrupole – FAME Standard Samples

For saturated FAME standards, a similar difference in fragmentation patterns between EI and LP-PCI modes was obtained from the single quadrupole (Fig. 6). For unsaturated FAMES, the MH⁺, [MH-32]⁺ and [MH-32-18]⁺ fragments remained present in the single quadrupole spectra, although the [M+54]⁺ ion was not present as it was in the ion trap (Fig. 7).

FIGURE 3. Close-up view of the LP-PCI acetonitrile fused silica line and GC capillary columns positioned through the GC transfer line



FIGURE 4. Comparison of EI (top TIC) and LP-PCI (bottom TIC) saturated FAME standards from ITQ 1100 ion trap. EI spectra show much fragmentation, while LP-PCI spectra show [M+1]⁺ and [M+40] adduct ions.



Seed Extract Spectra

A mixture of saturated and unsaturated FAMES were extracted and esterified from a single seed of *Camelina sativa* and run in both EI and LP-PCI modes using the ITQ 900 ion trap. Data from the extracts is shown in Fig. 8 and verifies the typical spectra seen in EI and LP-PCI modes.

FIGURE 5. Comparison of EI (top TIC) and LP-PCI (bottom TIC) unsaturated FAME standards from ITQ 1100 ion trap. The C:23 FAME (MWT 342) at 20.4 minutes contains 6 unsaturated bonds. The LP-PCI spectrum demonstrates the MH⁺, [MH-32]⁺, [MH-32-18]⁺ and [M+54]⁺ ions as well as the [M+40] adduct.

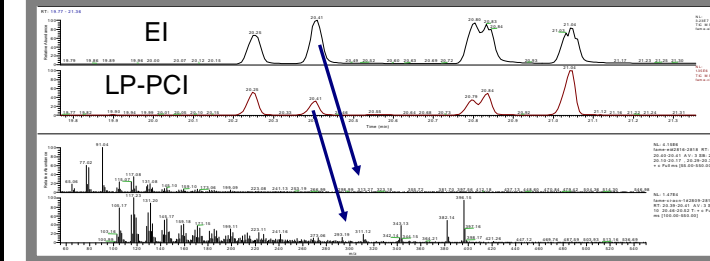


FIGURE 6. Example of a saturated FAME standard sample obtained from EI (top TIC) and LP-PCI (bottom TIC) modes with the DSQII single quadrupole MS. LP-PCI spectra show [M+1]⁺ and [M+40] adduct ions.

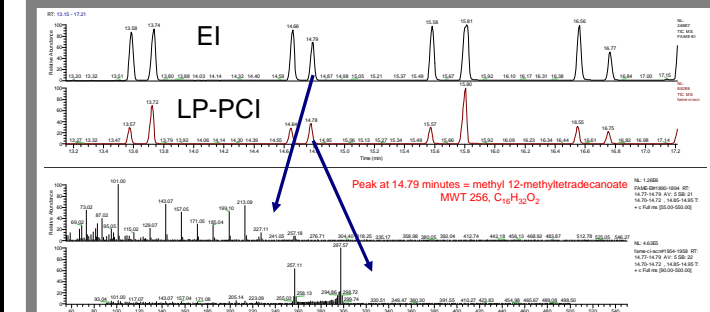


FIGURE 7. Example of an unsaturated FAME standard sample obtained from LP-PCI mode with the DSQII single quadrupole MS. LP-PCI spectra show [M+1]⁺, [M-32]⁺, [M-32-18]⁺ and a small amount [M+40] adduct ions.

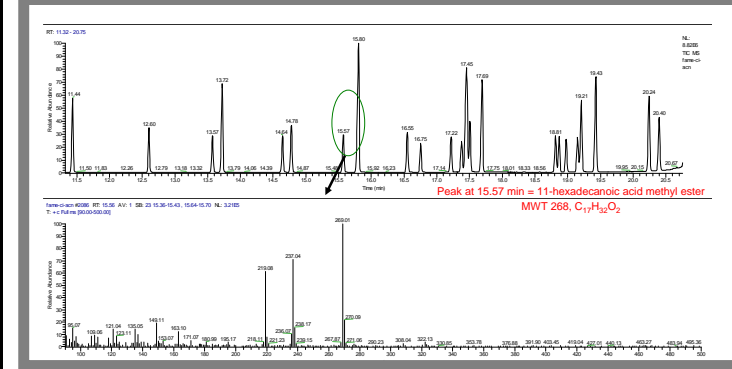
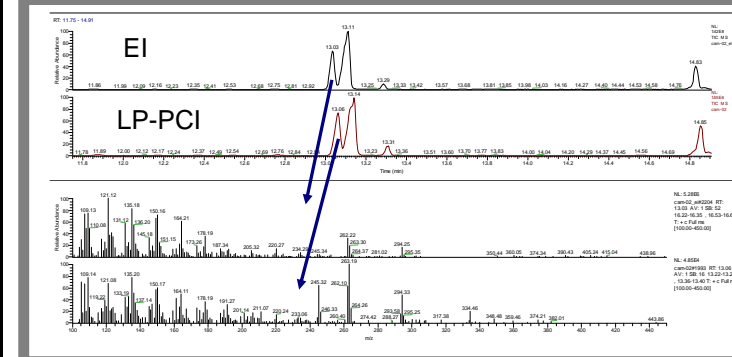


FIGURE 8. Example of an unsaturated FAME at 13.03 minutes from seed extract, *Camelina sativa*, MWT 294. Top TIC and spectra was run on the ITQ 900 ion trap in EI mode, while the bottom TIC and spectra was run on the same instrument in LP-PCI mode.



Conclusions

Acetonitrile LP-PCI has been shown to be a rapid method for identification of saturated and unsaturated FAMES. However, not all benchtop GC-MS systems are equipped to routinely run LP-PCI mode samples. We have demonstrated a simple, cost-effective setup for achieving LP-PCI that can be used with either ion traps or quadrupole instruments that provides selective, reliable data for characterizing FAME compounds.

References

- Moneti, G., Pieraccini, G., Dani, F.R., Catinella, S., Traldi, P., Rapid Communications in Mass Spectrometry (1996) 10:167.
- Midhaud, A.L., Diau, G., Abril, R., Brenna, J.T. Analytical Biochemistry (2002) 307:348-360.
- Midhaud, A.L., Yurawecz, M.P., Delmonte, P., Cori, B.A., Bauman, D.E., Brenna, J.T. Analytical Biochemistry (2003) 75:4925-4930.

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