# Authenticity control of olive oils by triglycerides LC analysis: method improvement using charged aerosol detection

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## INTRODUCTION

Extra virgin olive oil represents a corner stone of the healthy Mediterranean diet. It is the edible oil with the highest nutritional and sensory quality and consequently the most expensive among them. Because of these issues, it's the edible oils that more frequently undergoes to attempt of frauds and for this reason, continuous efforts have been devoted to the development of new and improved analytical methods able to detect emerging and sophisticated frauds.
The analysis of triacyclglycerols had been validated by means of the evaluation of the consistency of the theoretical composition calculated on the basis of the fatty acids composition and the experimental one, obtained by HPLC analysis, the difference, named ΔECN42 is suitable for detection of small amount of seed oil mixed with olive oil. However, from an analytical point of view, the use of refractive index detector for triglycerides analysis, which is recommended by European legislation and the IOC, prevents gradient elution separations, thus leading to incomplete TAGs resolution and overlapping peaks.
Over the past few years, charged aerosol detection has become a widely used technology in the pharmaceutical laboratory. The charged aerosol detector (CAD) generates a signal in direct proportion to the quantity of analyte present. Furthermore, it can be used with gradient elution, thus representing a potential alternative to refractive index detector.
In the present study, ultrahigh pressure liquid chromatography coupled with CAD detection was applied to the analysis of extra-virgin olive oil triglycerides. Different chromatographic separations under gradient elution were proposed and optimized using C30 and C18 reversed-phase columns. The performance of CAD detector for trilinolein (LLL) analysis was deeply investigated (linearity, repeatability, response uniformity, etc.).
Finally, the proposed UHPLC-CAD methodology has been also applied to the determination of trilinolein in extra virgin olive oil mixed in different proportions to simulate the adulteration of olive oil with high oleic sunflower oil, palm olein and a mix of them at different percentages of 2, 4, 6, 8 and 10 %.

## INSTRUMENTATION

**Sample preparation** (1)

Silica SPE cartridge (1 g, 6 ml)

Loading:
0.24 g/mL of oil dissolved in *n*-hexane

Washing:
6 mL of *n*-hexane

Elution:
10 mL of *n*-hexane/diethyl ether (87/13, v/v)

**Thermo Scientific™ UltiMate™ 3000 UHPLC System**

2.6 µm

**Thermo Scientific Accucore C18**
**2.1 x 150 mm**

**0.5 mL/min**

Mobile phase A= acetone/isopropanol (70/30, v/v)
Mobile phase B= acetonitrile

T column: 20 °C
V inj= 1ul
Injecting solution= SPE eluate

**250 bar**

| Time | Flow ml/min | %A | %B |
|---|---|---|---|
| 0 | 0,5 | 45 | 55 |
| 2 | 0,5 | 45 | 55 |
| 12 | 0,5 | 50 | 50 |
| 12 | 0,7 | 50 | 50 |
| 27 | 0,7 | 60 | 40 |
| 27 | 0,7 | 65 | 35 |
| 28 | 0,5 | 45 | 55 |
| 35 | 0,5 | 45 | 55 |

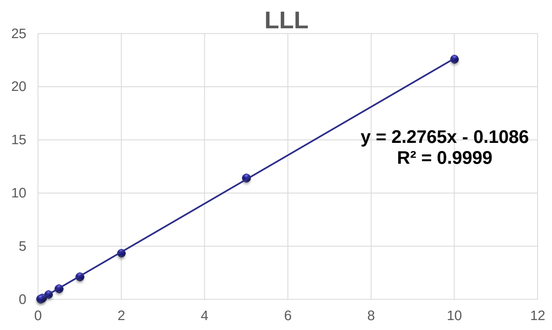
| CAD parameters | |
|---|---|
| Evaporator temperature: 50 °C | Data collection rate: 10 Hz |
| Filter: 3,6 | Power function: 1,65 |

**Thermo Scientific™ Corona™ Veo™ Charged Aerosol Detector**

## CAD DETECTOR

### Performance of CAD detector for *trilinolein*: ***Linearity***

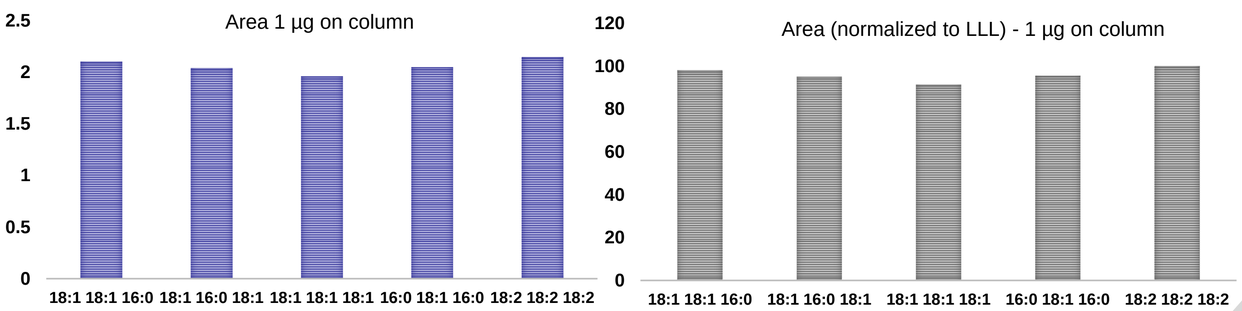
| LLL | pw 1,65 |
|---|---|
| µg on column | Area pA*min |
| 10 | 22,602 |
| 5 | 11,427 |
| 2 | 4,348 |
| 1 | 2,133 |
| 0,5 | 1,001 |
| 0,25 | 0,481 |
| 0,1 | 0,118 |
| 0,05 | 0,043 |




### Performance of CAD detector for trilinolein: ***Repeatability***

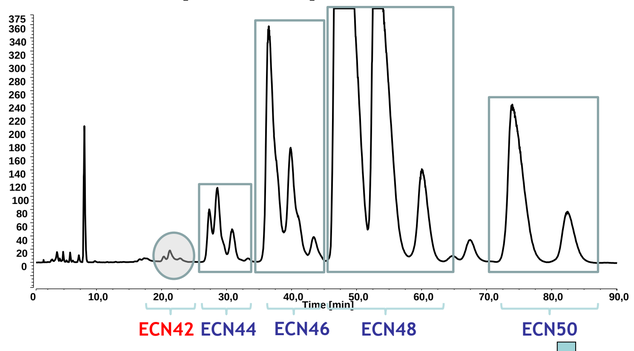
| Repeatability *n=9* | | | |
|---|---|---|---|
| | Mean, area pA*min | RSDr | RSD% |
| STD LLL (7 µg on column) | 4,606 | 0,187 | 4,1 |
| STD LLL (0,75 µg on column) | 0,528 | 0,022 | 4,2 |
| Pomace oil | 0,186 | 0,007 | 3,9 |

### Performance of CAD detector: ***Uniformity of CAD to different TAGs species***




## UHPLC

**EVOO - low linoleic acid content 3.5%**
**Isocratic elution [IOC method (1)]**




5 µm

C18
4.6 x 250 mm
1.5 mL/min

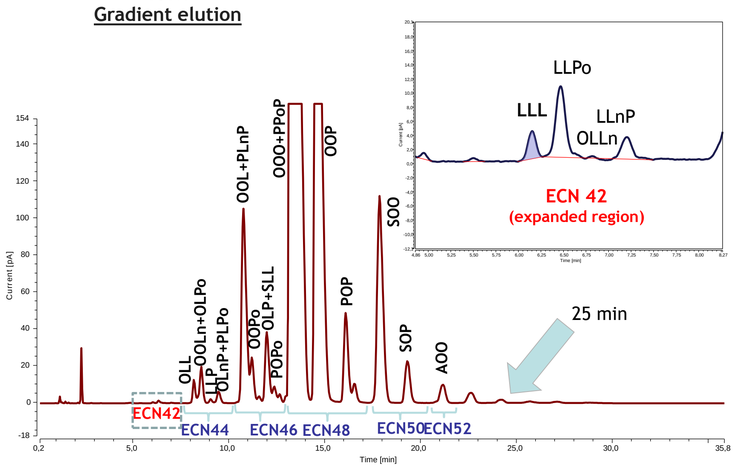
96 bar

| Time | Flow mL/min | % A | % B |
|---|---|---|---|
| 0 | 1.5 | 50 | 50 |
| 90 | 1.5 | 50 | 50 |

Mobile phase A= acetone
Mobile phase B= acetonitrile

T column: 25 °C
V inj= 10 µl
Injecting solution= SPE eluate

**EVOO - low linoleic acid content 3.5%**
**Gradient elution**




Accucore C18
2.1 x 150 mm
0.5 mL/min
2.6 µm
250 bar

| Time | Flow ml/min | %A | %B |
|---|---|---|---|
| 0 | 0.5 | 45 | 55 |
| 2 | 0.5 | 45 | 55 |
| 12 | 0.5 | 50 | 50 |
| 12 | 0.7 | 50 | 50 |
| 27 | 0.7 | 60 | 40 |
| 27 | 0.7 | 65 | 35 |
| 28 | 0.5 | 45 | 55 |
| 35 | 0.5 | 45 | 55 |

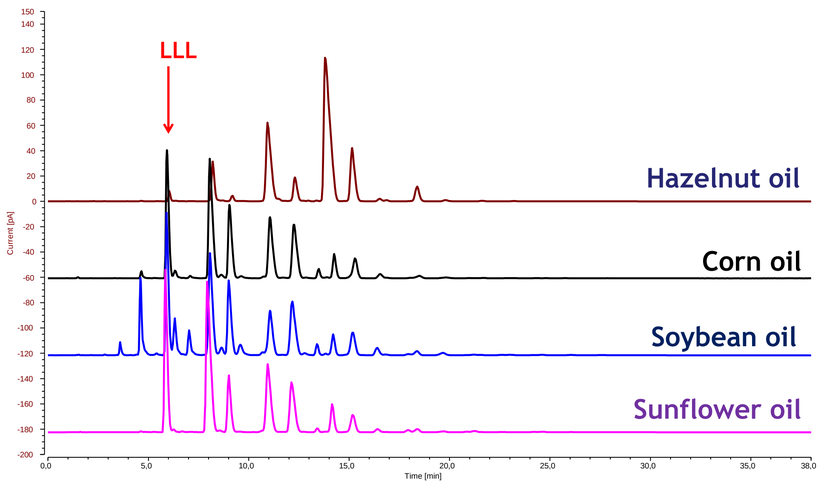
Mobile phase A= acetone/isopropanol (70/30, v/v)
Mobile phase B= acetonitrile

T column: 20 °C
V inj= 1ul
Injecting solution= SPE eluate

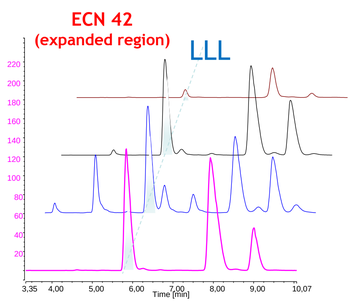
CORONA:
Power Function: 1,65
Data Collection rate: 10 Hz
Filter: 3,6
Evaporation Temperature= 50 °C

## *UHPLC-CAD chromatograms of other common vegetable oils*

other vegetable oils




Accucore C18
2.1 x 150 mm
0.5 mL/min
2.6 µm
250 bar



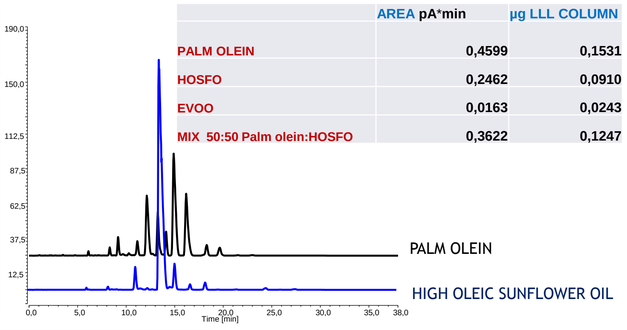

## SAMPLES ANALYSIS - *adulterated olive oil*

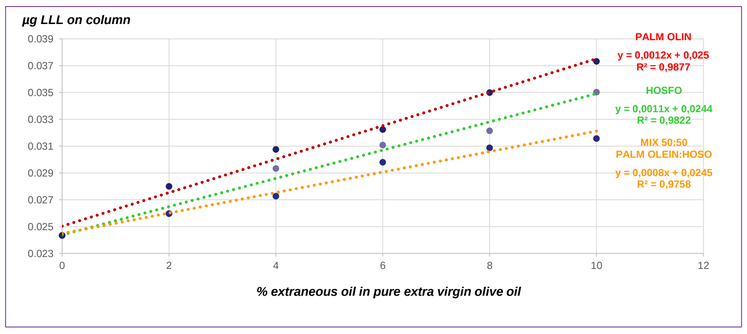
Pure EVOO with low linoleic acid content (3,5 %)

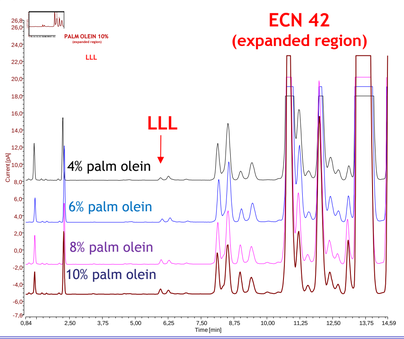
*mixed with*

| Palm olein | High oleic sunflower oil | Mix 50:50 |
|---|---|---|
| 2% | 2% | 2% |
| 4% | 4% | 4% |
| 6% | 6% | 6% |
| 8% | 8% | 8% |
| 10% | 10% | 10% |

| | AREA pA*min | µg LLL COLUMN |
|---|---|---|
| PALM OLEIN | 0,4599 | 0,1531 |
| HOSFO | 0,2462 | 0,0910 |
| EVOO | 0,0163 | 0,0243 |
| MIX 50:50 Palm olein:HOSFO | 0,3622 | 0,1247 |




## CONCLUSIONS

- Differently from RI detector, CAD allows the use of gradient elution, thus improving separation of the PN42 TAGs group;
- The use of UHPLC-CAD allows to obtain a good separation and quantification of trilinolein;
- CAD has good linearity for trilinolein over a wide range from 50 to 10.000 ng injected on column;
- The results indicate that CAD shows good precision and accuracy;
- CAD showed very high response uniformity for triglycerides with different number of C atoms and/or double bonds;
- The UHPLC-CAD procedure developed has proved capable of separating TAGs in a reduced time and with a much lower consumption of mobile phases when compared to the official method;
- The proposed UHPLC-CAD method is able to detect small increases in trilinolein content in extra virgin olive oil adulterated with 2, 4, 6, 8 and 10 % of high oleic sunflower oil, palm olein and a mix of them.

## REFERENCES

(1) International Olive Council. (2001). Determination of the difference between actual and theoretical content of triacylglycerols with ECN 42. COI/T.20/Doc. No. 20 Rev. 3. Madrid, Spain.