PharmaGnose

LABORATORY REPORT

SUBJECT: Qualitative and quantitative analysis of polyphenols in olive oil sample

DATE: 21/02/2025

SAMPLE	
NAME	**INFORMATION**
OLE_1814	Olive Oil Sample "**SAMPLE A – eladaki tou voria**"

ANALYSIS

A. Extraction of biophenols
B. HPLC-DAD Analysis – profiling
C. Qualitative and quantitative analysis of the major olive oil biophenols
D. Verification of EFSA's approved health claim in the olive oil sample

OLIVE OIL SAMPLE INFORMATION

SAMPLE CODE	**OLE_1814 "SAMPLE A – eladaki tou voria"**
PRODUCER NAME	**GEORGE KSEROUDAKIS**

Aim of the study

The main goal of this study is to determine the major biophenols in olive oil, such as Hydroxytyrosol, Tyrosol, Oleacein, Oleocanthal and to investigate the EFSA's approved health claim requirements in the olive oil sample. Olive oil is a complex and multifaceted mixture of compounds and a source of valuable nutrients. In 2012, the European Food Safety Authority (EFSA), assessing the biological properties of olive oil phenolic components issued a scientific opinion in favor of the specific health claim, pointing out that olive oil polyphenols contribute to the protection of blood lipids from oxidative damage (Regulation No 432/2012 of EC). Characteristic compounds of olive oil are hydroxytyrosol, tyrosol as well as the secoiridoidsoleacein, oleocanthal, oleuropein and ligstroside aglycons. Many analytical methods have been proposed in literature for the determination of the phenolic components as well as the verification of the EFSA's health claim. The only recognized method is the one proposed by the International Olive Council (IOC).

A. EXTRACTION / SAMPLE PREPARATION

In order to isolate polar phenolic components from the **OLE_1814** olive oil sample, a liquid-liquid extraction method assisted by centrifugation and ultrasound technique was applied (*Determination of biophenols in olive oils by HPLC-COI-/T.20/Doc No 29*). Specifically, in a 10 ml test tube, 2.0 g of olive oil and 1 ml of the internal standard solution (syringic acid) were added and vortexed for 30 sec. Then, 5 ml of the methanol/water (80:20 v/v) were added and vortexed for a further minute. The mixture was placed in an ultrasonic bath for 15 min at room temperature and then centrifuged for 25 min. After, an aliquot of the supernatant phase was taken, filtered and forwarded for HPLC analysis.

B. HPLC-DAD ANALYSIS

The High-Performance Liquid Chromatography technique coupled to a photodiode array detector (HPLC-DAD) was used for (a) the qualitative determination of biophenols in olive oil (figures 1&2), (b) the quantitative determination of major olive oil biophenols (Table 2) and (c) the determination of olive oil biophenols, referred to EFSA's approved health claim (Table 3). The proposed IOC method applied was performed according to analytical conditions referred to COI / T.20 / Doc No 29 method (International Olive Council (IOC) 2009). Specifically, the separation was achieved on a reversed-phase Spherisorb Discovery HS C18 column (250 × 4.6 mm, 5μm; Supelco) using a mobile phase consisting of 0.2% aqueous orthophosphoric acid (A) and methanol/acetonitrile (50:50 v/v) (B), at a flow rate of 1.0 mL/minute and ambient temperature. The injection volume was held constant at 20μl. The applied gradient elution was as follows: 0 min, 96% A and 4% B; 40 min, 50% A and 50% B; 45 min, 40% A and 60% B; 60 min, 0% A and 100% B; 70 min, 0% A and 100% B; 72 min, 96% A and 4% B; 82 min, 96% A and 4% B. Chromatograms were monitored at 280 nm.

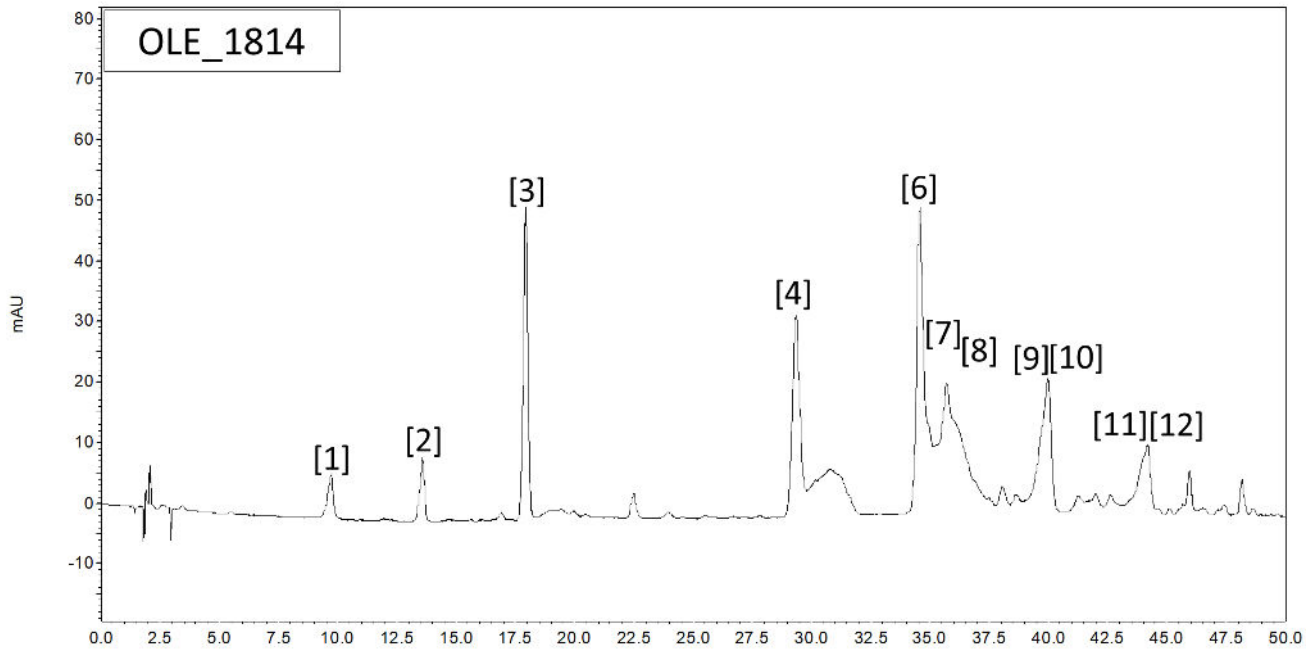

Figure 1: RP-HPLC-DAD chromatogram of the analyzed **OLE_1814** sample at 280 nm. The identified compounds are highlighted.

C. RESULTS

Qualitative determination of biophenols

The qualitative determination of the phenolic components was carried out using an analytical standard working solution consisted of 12 (**see Table 1**) out of 27biophenols referred to the protocol of IOC method. Some of them were commercially available and the others were isolated by olive oil. In figures 1, 2 are highlighted olive oil biophenols which were identified in the **OLE_1814** sample.

Table 1: Standard phenolic compounds related to the EFSA health claim *.

[1] Hydroxytyrosol
[2] Tyrosol
[3] Syringic acid (Internal Standard)
[4] Oleacein
[5] Oleuropein
[6] Oleocanthal
[7] Pinoresinol
[8] 1-acetoxy-pinoresinol
[9] Oleuropein aglycon
[10] Luteolin
[11] Apigenin
[12] Ligstroside aglycon
Vanillic acid
Vannilin
p-coumaric acid
o-coumaric acid
Ferulic acid
Hydroxyltyrosol acetate
Tyrosol acetate

* In Table 1 are presented 19 of the 27 phenolic compounds that are referred to the EFSA health claim. The rest ones are not mentioned because there are no commercially available standards.

Quantitative determination of the major olive oil biophenols
(Data available on request).

Table 2: Quantitative determination of Hydroxytyrosol (HT), Tyrosol (T), Oleacein (OLEA) and Oleocanthal (OLEO) in the **OLE_1814** olive oil extract

Sample Code	HT (mg/Kg OO)	T (mg/Kg OO)	OLEA (mg/Kg OO)	OLEO (mg/Kg OO)
OLE_1814 (n=3)	**2.84**	**9.16**	**69.80**	**166.17**

Determination of total biophenols, referred to EFSA's approved health claim
Biophenol content (lignans, flavonoids, phenolic acids, secoiridoids, oxidative forms of oleuropein and ligstroside aglycones), expressed in mg Tyrosol / kg VOO, was estimated by measuring the sum of the areas of the related chromatographic peaks (figures 1&2) and the relative response factor of external standard solutions of tyrosol and syringic acid was calculated (RRF syringic acid/ tyrosol), as described in the COI / T.20 / Doc No 29 method.

Table 3: Quantitative determination of phenolic components referred to EFSA's approved health claim of the analysed **OLE_1814** sample.

Sample Code	RRF*	Determination of olive oil biophenols (mg Tyr/Kg olive oil)
OLE_1814	**4.86**	**361**

RRF*: Relative Response Factor for the expression of the result as Tyrosol.

MAJOR OBSERVATIONS AND COMMENTS

- As shown in the chromatogram at 280 nm (**Figure 1**), which is an indicative wavelength for the detection of phenyl alcohols, **OLE_1814** olive oil sample was found to contain **2.84 mg Hydroxytyrosol / kg olive oil**, **9.16 mg Tyrosol / kg olive oil**, **69.80 mg Oleacein** and **166.17 mg Oleocanthal / kg olive oil**.

  It is worth mention that Oleacein and Oleocanthal are secoiridoid derivatives of Hydroxytyrosol and Tyrosol respectively, which occur in high levels in the polyphenol fraction of extra virgin olive oil. They can provide the Hydroxytyrosol / Tyrosol units and recent studies associate these compounds with strong anti-inflammatory activity.

- Moreover, the **OLE_1814** polyphenolic extract of the tested sample is characterized by the presence of other bioactive compounds, such as flavonoids (e.g. luteolin, apigenin), lignans (e.g. pinoresinol, acetoxypinoresinol) and other secoiridoids **(Figure 1)**.

- Analyzing the **OLE_1814** sample by the **IOC method**, it was found that, the date of analysis, the total biophenols in the tested sample is **361, expressed as mg Tyr per Kg OO**. This founding means that the **OLE_1814** analyzed olive oil sample **meets the specifications of the EU regulation 432/2012 and can bear the health claim approved by EFSA (http://www.gcsl.gr/media/trofima/reg-432-2012.pdf), since it contains > 5 mg Hydroxytyrosol and derivatives/20g olive oil.**

PharmaGnose,

Analytical Department

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