Project co-financed by the European Regional Development Fund

ARISTOIL

"Reinforcement of Mediterranean olive oil sector competitiveness through development and application of innovative production and quality control methodologies related to olive oil health protecting properties"

PRIORITY AXIS 1: Promoting Mediterranean innovation capacities to develop smart and sustainable growth

OBJECTIVE: 1.1 To increase transnational activity of innovative clusters and networks of key sectors of the MED area

Project website: http://aristoil.interreg-med.eu/

DELIVERABLE Number: 3.1.2
Title of DELIVERABLE: Automated measurement application

ACTIVITY n. 3.1: Development of methodology for monitoring olive oil quality

WP n. 3: STUDYING

PARTNER IN CHARGE: National and Kapodistrian University of Athens, Department of Pharmacognosy and Natural products Chemistry (UoA)

PARTNERS INVOLVED: 1. National and Kapodistrian University of Athens, Department of Pharmacognosy and Natural products Chemistry (UoA), 2. ARISTOLEO (ARI)

Status:

Draft □	*Final* ×	*Version n. 01*

Distribution:

Confidential ×	*Public* □	

Date: 31 December 2017

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Contents

I. Automated measurement application

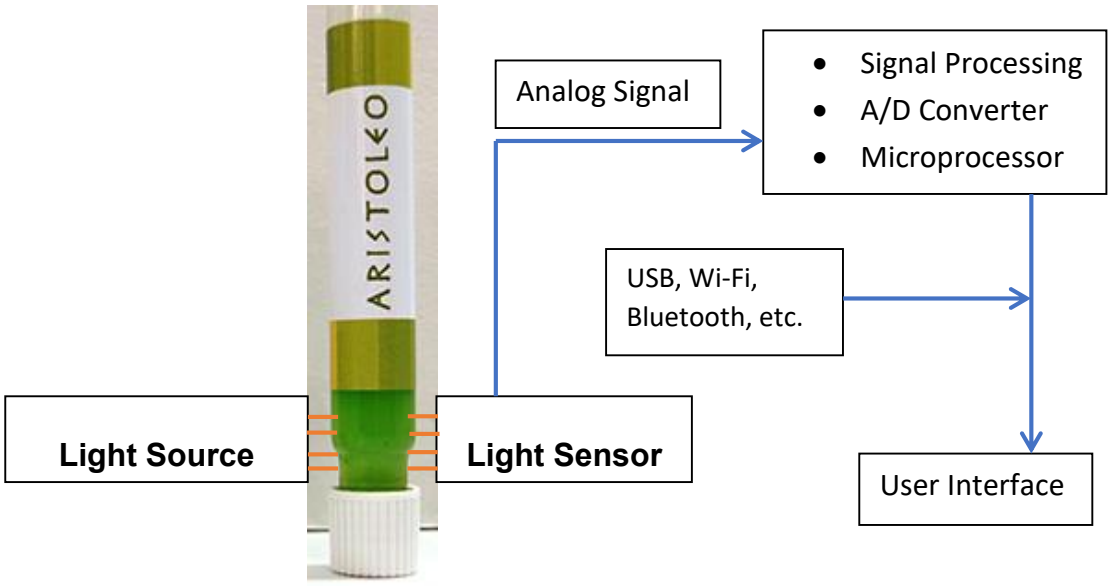
General description: A prototype of an instrument permitting automated colorimetric measurement has been constructed. It also includes a smart phone application for visual recognition of colorimetric results.

1. Method development

ARISTOLEO tested the specificity of the previously developed method in order to guarantee that the obtained measurement is associated only with the target compounds. The colorimetric aristoleo method is measuring only the levels of oleocanthal and oleacein and is alternative cheap way of measurement. It was found that the method is selective for the two compounds and that there is no interaction with other similar compounds.

Parameters that were investigated during the optimization phase included temperature, time, wavelength of measurement as well as reagents concentration and the need or not for centrifugal separation at the last step before the optical measurement.

After the optimization of the colorimetric methodology an automated instrument for accurate measurement was developed by ARI, having the name **Aristometro**. The instrument includes a monochromatic light source and a detector of light absorption. The instrument was calibrated using the pure standards after addition to zero phenolic content olive oil. The calibration curves provided equations that correlated the light absorption with the phenolic concentration. The instrument has been programmed to afford automatically the phenolic content (Oleocanthal+Oleacein content; D1 index).



The above Figure illustrates the design characteristics of the Aristometro. A Light Source sheds light on the Aristoleo® sample. Part of this light is absorbed by the sample and the rest passes through it and is detected by a Light Sensor, producing an electronic Analog Signal. The electronics of the Aristometer™ process this signal and with the help of an nterface sends the measurement to a user interface Liquid Crystal screen. The User Interface applies to the measurement the appropriate calibration curves and displays the measured amount of oleocanthal and oleacein found in olive oil.

The ARISTOLEO method which is used for the measurement of the sum of oleocanthal and oleacein in olive oil is based on the correlation between the intensity of the green color that appears on the aqueous phase and the concentration of oleocanthal and oleacein.

Aristometro is a device which was constructed for the measurement of absorption at 639nm in samples of olive oil in which we have implied the method of optical measurement of oleocanthal and oleacein (ARISTOLEO). Aristometro simplified the method of quantitative measurement of the sum of oleocanthal and oleacein.

The final method of optical measurement of the sum of oleocanthal and oleacein (D1 ratio) is the following:

- 7,5 ml of olive oil is put in the vial
- 1,5ml of reagent (p-hydroxyanthranilic acid) which is dissolved in acetic acid is added
- We agitate the sample for 30sec making sure that it becomes homogeneous and we wait for 30sec
- 1,5ml of distilled water is added and the vial is vigorously agitated for 30 sec
- We wait for 30min, we put the sample in the ARISTOMETRO and the concentration of the sum of oleocanthal and oleacein is automatically calculated.

VIDEO DEMONSTRATIONS OF THE ARISTOMETRO CAN BE FOUND AT:

https://drive.google.com/drive/folders/1ldMlrllN0tHgDhnQvXdfXFvr9TkHORpd

Figure 1: Depiction of ARISTOMETRO

The method of ARISTOLEO was validated using a zero phenolic sample of olive oil in which we added specific amounts of oleacein and oleocanthal, obtaining the following concentrations 0, 50, 150, 250, 500, 750, 1000, 1250 and 1500 mg/kg. After the waiting time, the phases were separated and we measured the absorption in the aqueous phase using a laboratory spectrophotometer and ARISTOMETRO.

C(mg/kg)	A(639nm) using spectrophotometer	ARISTOMETRO C(mg/kg)	
0	0	0	47680
50	32	57	43980
150	94	156	37483
250	157	241	32527
500	313	527	20419
750	470	760	13547
1000	626	1020	9002
1250	783	1260	6001
1500	940	1510	4029

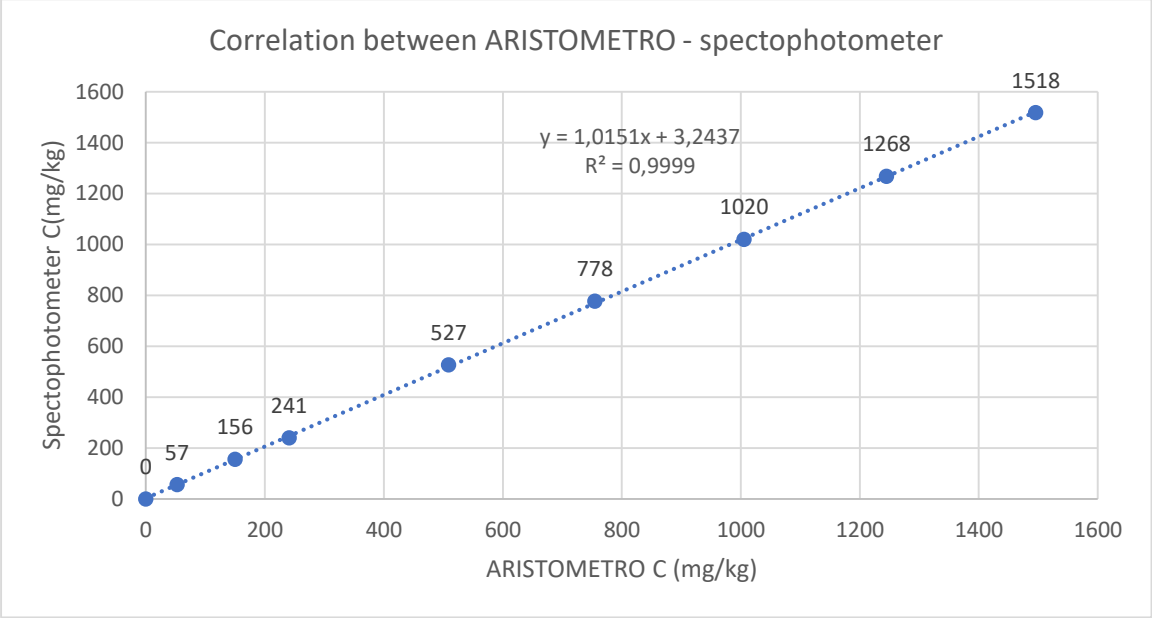


Diagram 1: Correlation of the sum of oleocanthal and oleacein using ARISTOMETRO and spectrophotometer

Oleocanthal and oleacein was added in specific concentration in olive oil , the optical measurement of the D1 ratio was applied and the absorptions was measured using ARISTOMETRO .

C(mg/kg) oleocanthal and oleacein	ARISTOMETRO C(mg/kg)
50	60
150	149
200	211
300	317
400	414
450	470
600	609
750	765
900	910
1050	1063
1200	1221
1350	1363
1500	1554

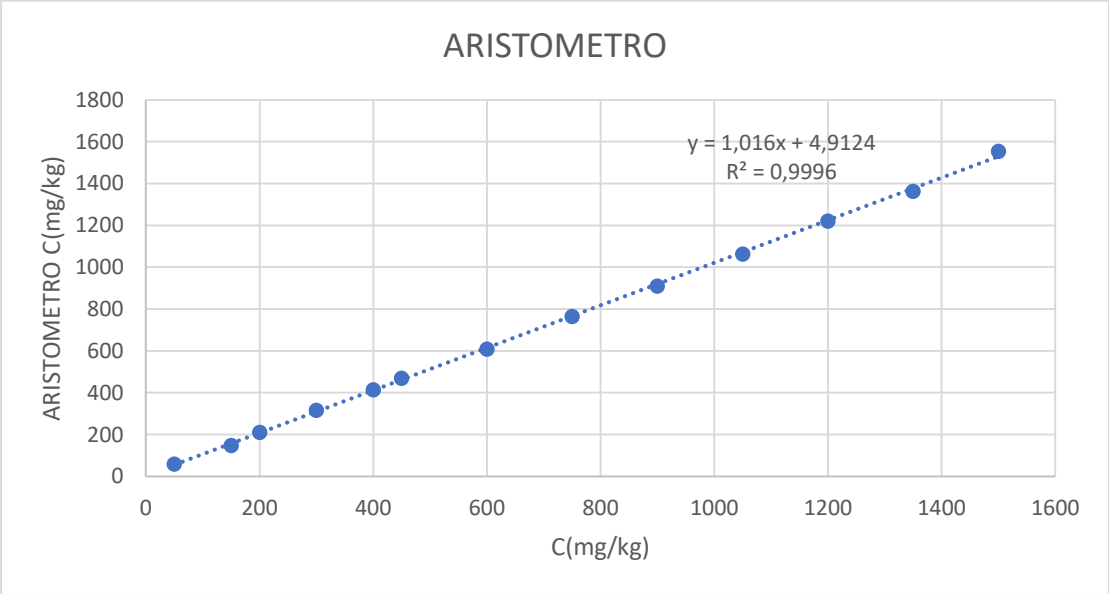


Diagram 2: Calibration curve of the sum of oleocanthal and oleacein using ARISTOMETRO

2. Calibration curves of olive oil phenolic compounds using the ARISTOMETRO

A. Calibration curve of oleacein

We weighted 23.5mg oleacein and dissolved it in 21,2g of olive oil with 0 concentration in phenolic compounds. Also we construct the following concentrations 0 ,150, 250, 500, 750 and 1000, 1250 and 1500ppm and in these samples the quantitative method was applied. Finally, the absorption was measured in 639nm using ARISTOMETRO.

The purity of the oleacein was identified by H1-NMR spectrum

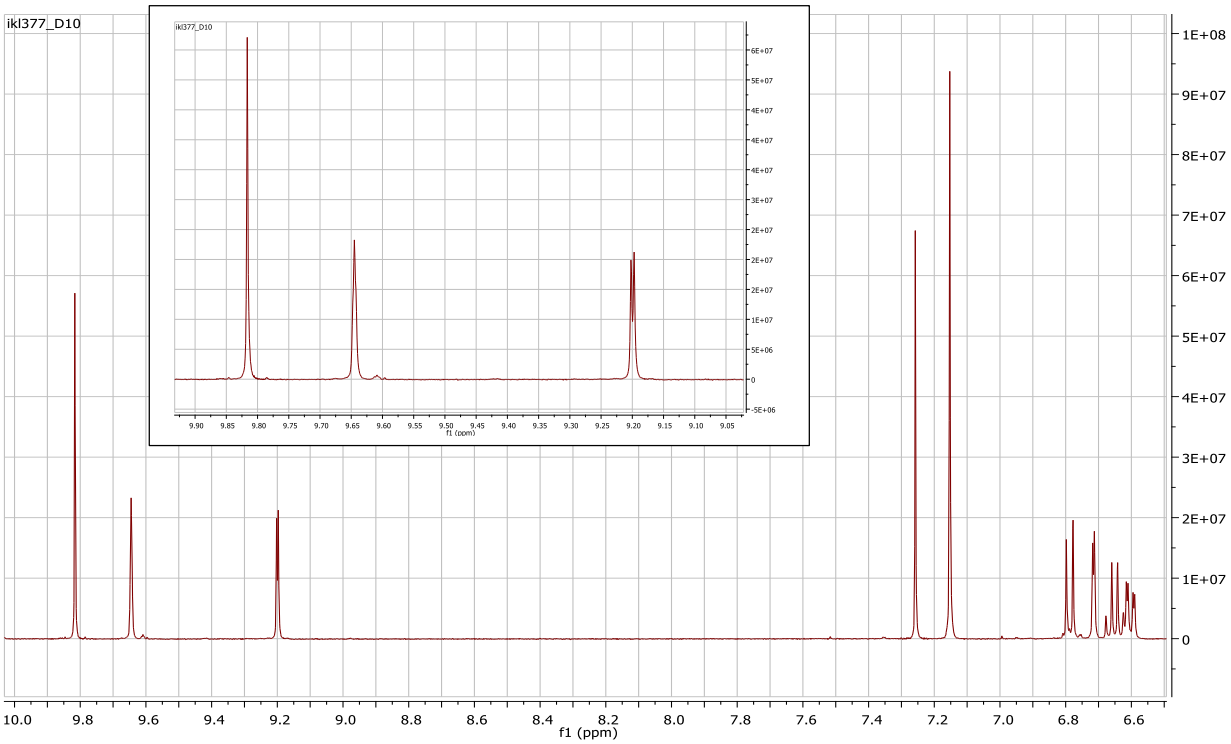


Figure 2: Depiction of the spectrum 1H-NMR of oleacein

C(mg/kg) (	C (mg/kg) ΑΡΙΣΤΟΜΕΤΡΟ
0	0
41.5	45
83	85
166	170
249	252
332	330
415	410
498	500
664	670
830	820
1037.5	1030
1245	1250

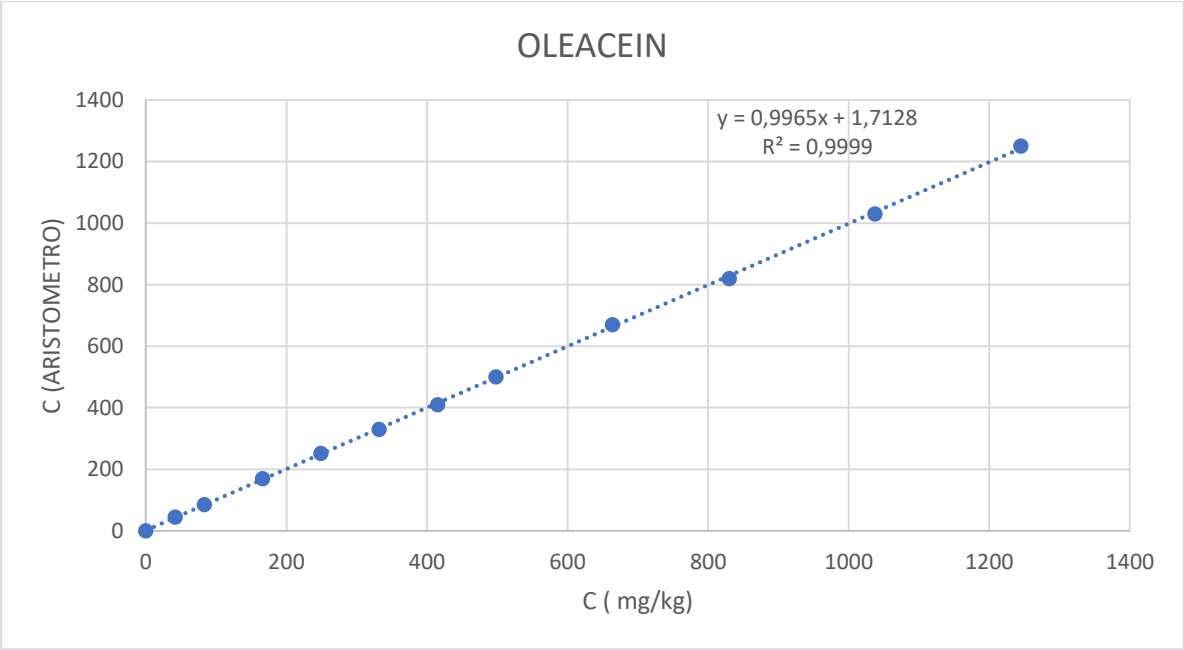


Diagram 3: Calibration curve of oleacein using ARISTOMETRO

B. Calibration curves of oleocanthal

We weighted 23.5mg oleocanthal and dissolved it in 21,2g of olive oil with 0 concentration in phenolic compounds. Also we construct the following concentrations 0 ,150 , 250 , 500 , 750 and 1000, 1250 and 1500ppm and in these samples the optical measurement of the sum of oleocanthal and oleacein was applied. Finally, the absorption was measured in 639nm using ARISTOMETRO.

The purity of the oleacein was identified by H1-NMR spectrum

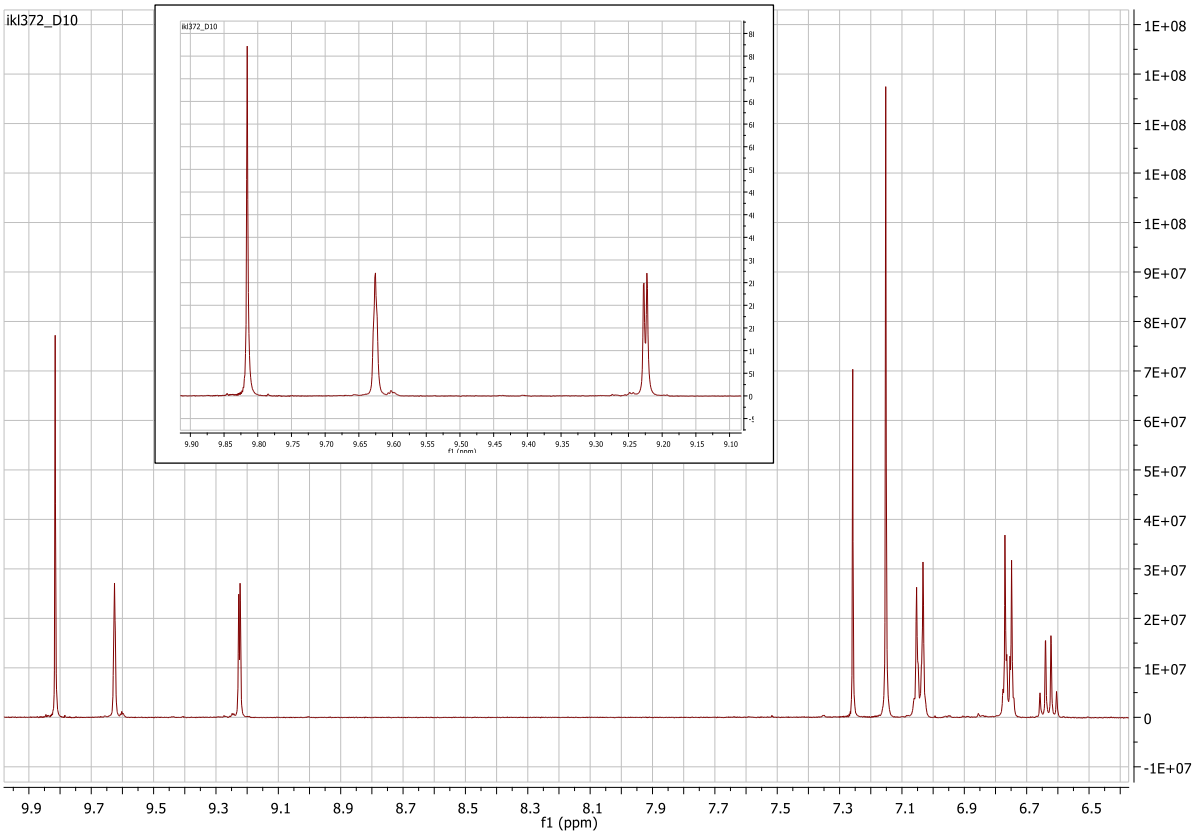


Figure 3: Depiction of the spectrum 1H-NMR of oleocanthal

C*(mg/kg)	C (mg/kg) ARISTOMETRO
0	0
39.5	42
79	85
158	165
237	230
316	325
395	407
474	485
632	650
790	800
987.5	1000
1185	1200

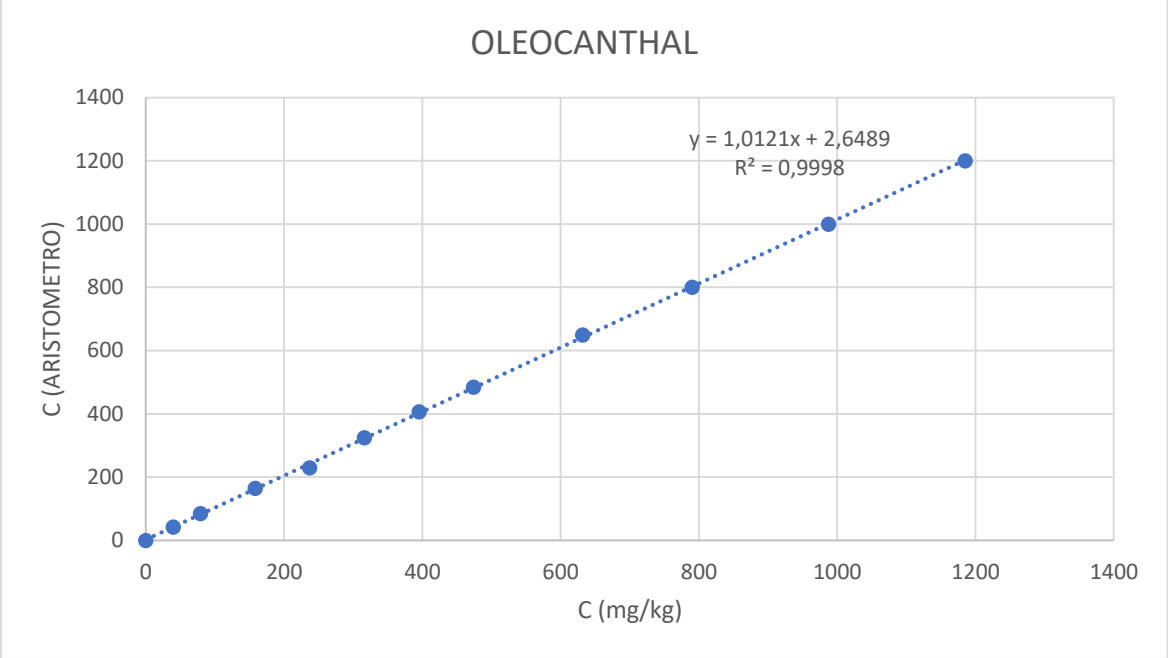


Diagram 4 : Calibration curve of oleocanthal using ARISTOMETRO

C. Calibration curves of oleomissional and oleoeuropeindial

We weighted 23.5mg oleomissional and oleoeuropeindial and dissolved it in 21,2g of olive oil with 0 concentration in phenolic compounds. Also we construct the following concentrations 0 ,150 , 250 , 500 , 750 and 1000ppm and in these samples the optical measurement of the sum of oleocanthal and oleacein was applied. Finally, the absorption was measured in 639nm using ARISTOMETRO.

ARISTMETRO was restarted by using distilled water.
The purity of the oleomissional was identified by H1-NMR spectrum

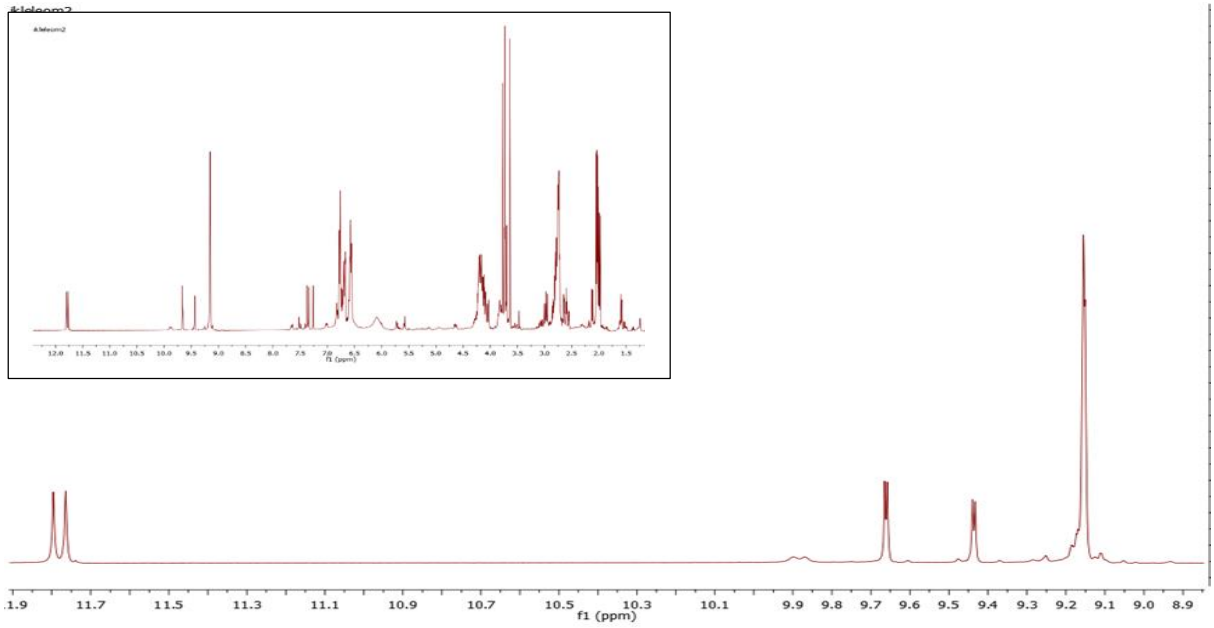


Figure 4: Depiction of the spectrum 1H-NMR of oleomissional and oleoeuropeindial

C(mg/kg)	C (mg/kg) ARISTOMETRO
0	0
100	2
200	1
400	0
500	4
600	4
800	3
1000	4

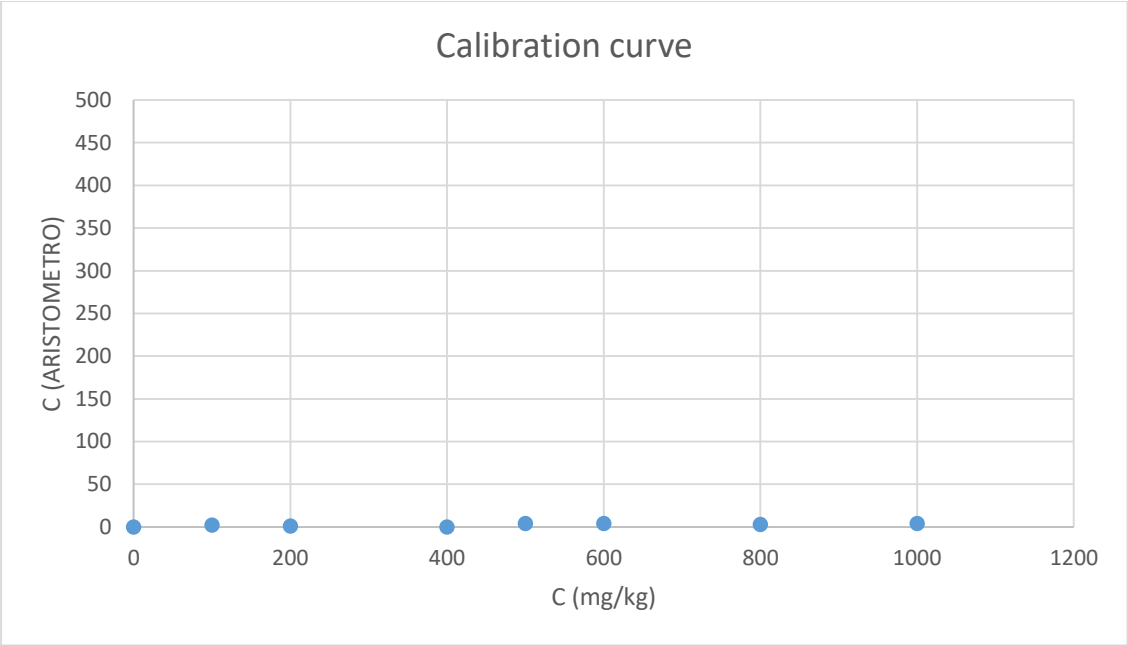


Diagram 5 : Calibration curve of the mix of oleomissional and oleoeuropeindial using ARISTOMETRO

It is obvious that the mix of oleomissional and oleoeuropeindial do not have absorption in 639nm.

D. Calibration curves of oleuropein aglycon (monoaldehyde form)

We weighted 23.5mg oleuropein aglycon (monoaldehyde form) and dissolved it in 21,2g of olive oil with 0 concentration in phenolic compounds. Also we construct the following concentrations 0 ,150 , 250 , 500 , 750 and 1000ppm and in these samples the optical measurement of the sum of oleocanthal and oleacein was applied. Finally, the absorption was measured in 639nm using ARISTOMETRO.

The purity of the oleacein was identified by H1-NMR spectrum

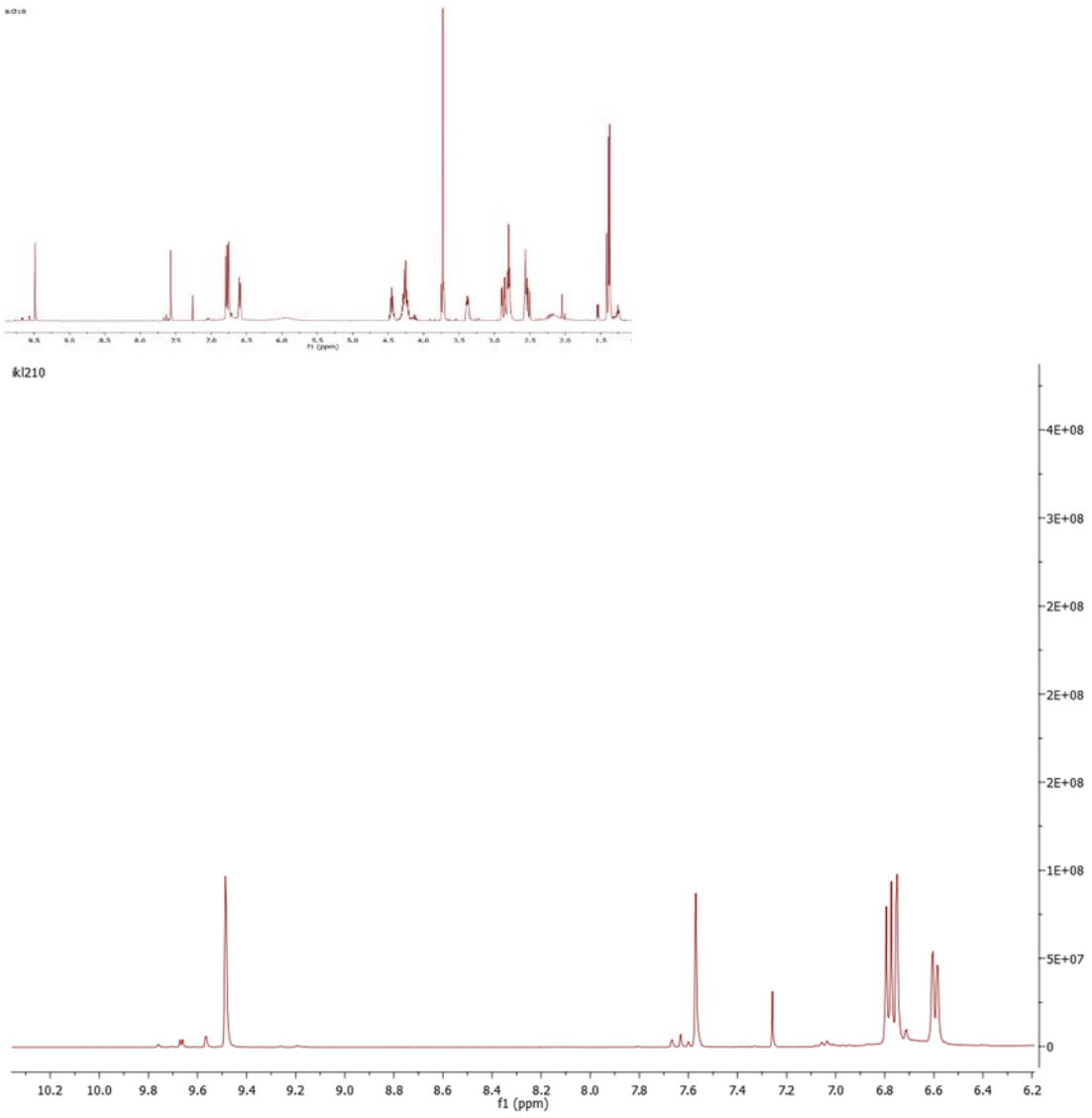


Figure 5 : Depiction of the spectrum 1H-NMR of oleuropein aglycon (monoaldehyde form)

C(mg/kg)	C (mg/kg) ARISTOMETRO
0	0

100	2
200	1
400	0
500	4
600	4
800	3
1000	4

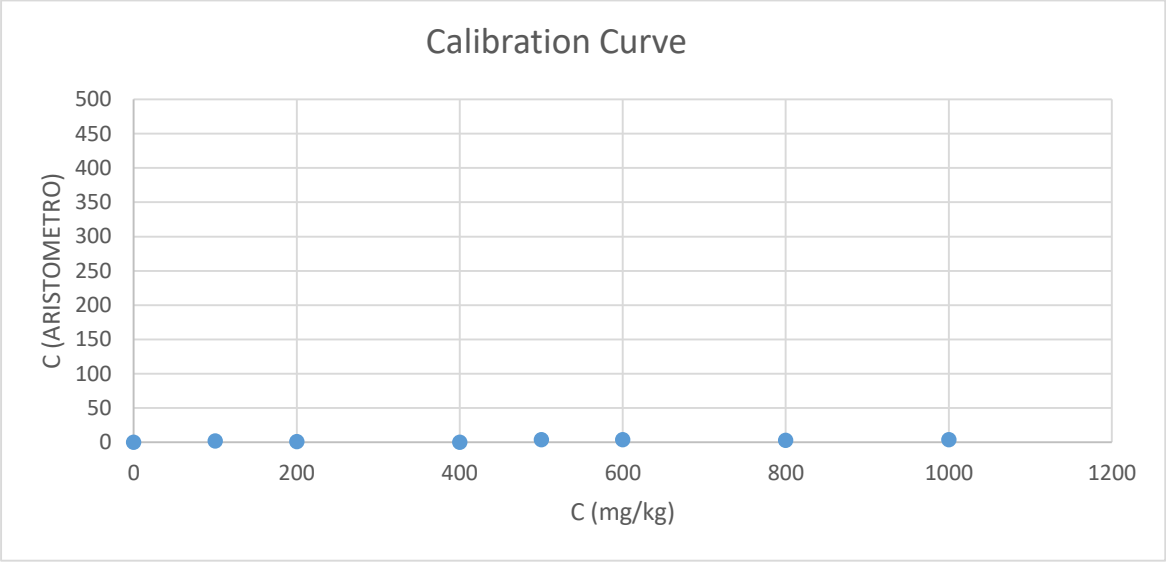


Diagram 6 : Calibration curve of the mix of oleomissional and oleoeuropeindial using ARISTOMETRO

It is obvious that the mix of oleuropein aglycon (monoaldehyde form) do not have absorption in 639nm.

E. Calibration curves of oleocanthalic acid

We weighted 23.5mg oleocanthalic acid **and** dissolved it in 21,2g of olive oil with 0 concentration in phenolic compounds. Also we construct the following concentrations 0 ,150, 250, 500, 750 and 1000ppm and in these samples the optical measurement of the sum of oleocanthal and oleacein was applied. Finally, the absorption was measured in 639nm using ARISTOMETRO.

The purity of the oleacein was identified by H1-NMR spectrum

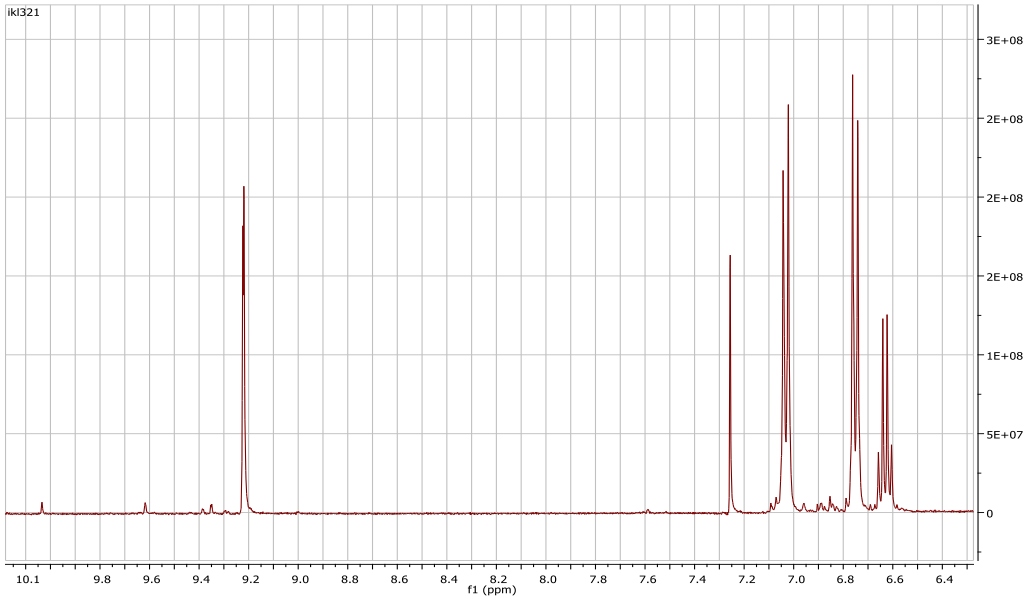


Figure 6: Depiction of the spectrum 1H-NMR of oleocanthalic acid

C(mg/kg)	C (mg/kg) ARISTOMETRO
0	0
100	2
200	1
400	0
500	4
600	4
800	3
1000	4

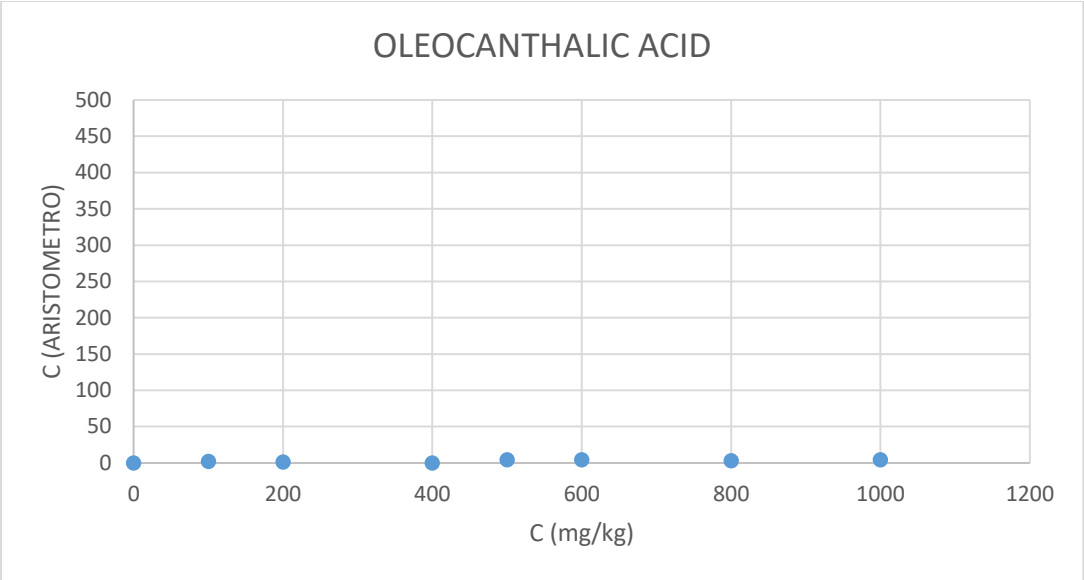


Diagram 7 : Calibration curve of oleocanthalic acid using ARISTOMETRO

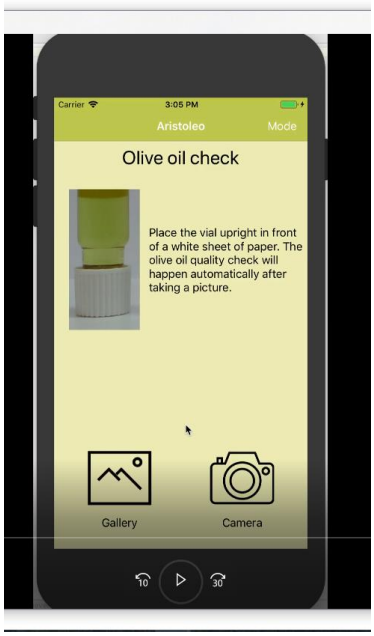
It is obvious that oleocanthalic acid do not have absorption in 639nm.

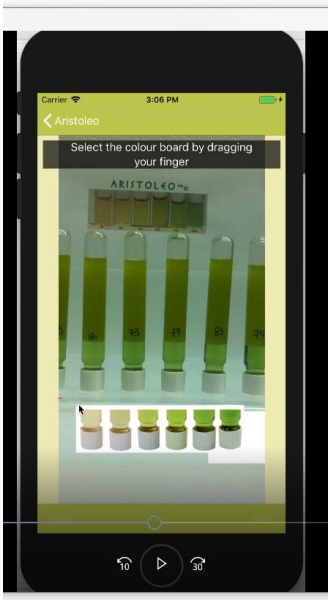
It is obvious that the reagent of ARISTOLEO method reacts only with oleocanthal and oleacein under acid conditions

II. Smart Phone Application

A smart phone application has been developed. The application is able to take a photo of the Aristoleo tube and automatically recognize the color and compare it with the printed index.

The way of use of the application is provided as a video demonstration.



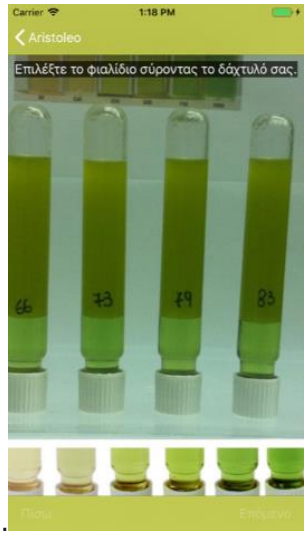


ARISTO APP INSTRUCTIONS OF USE

1. Open the AristoApp Press the camera icon.
2. Place the vial in front of the white piece of paper behind the colour chart. Take a photo. Press next.
3. Choose the colour in the vial you want to analyze. Grab cut the section of the colour chart and press next.
4. Choose the colour chart section you want to compare it to. Grab cut the colour board.
5. The combined total of oleocanthal and oleacein appears on the screen.
6. Compare the result on the screen with a visual inspection of the colour chart and the vial.
7. If it does not closely match repeat the process. Adjust the positioning of the vial to the light source and try again
8. The AristoApp learns as you learn to choose the best lighting conditions combined with the quality of your smartphone camera
9. There is a wide variability of lighting sources and smartphone camera lens quality and megapixels.
10. The AristoApp learns as you learn to choose the best lighting set up for your smartphone camera for the best results.

1. 

2. 
Carrier
1:18 PM
Aristoleo
Λειτουργία
Έλεγχος ελαιολάδου
Τοποθετήστε το φιαλίδιο κάθετα μπροστά από μια λευκή κόλλα χαρτί. Κάνοντας λήψη φωτογραφίας θα γίνει ο αυτόματος έλεγχος της ποιότητας του ελαιολάδου.
Επιλογή Τρόπου Λειτουργίας
GrabCut
Grab Cut
Overlay: 6 χρώματα
Overlay: 12 χρώματα
Άκυρο

3. 
Carrier
1:18 PM
Aristoleo
Επιλέξτε το φιαλίδιο σύροντας το δάχτυλό σας.

4. 
Carrier
10:35 AM
Aristoleo
Αποτελέσματα
Άθροισμα ελαιοκανθάλης και ελαιασίνης 750-1000mg/kg