A Non-Targeted High-Resolution Mass Spectrometry study for extra virgin olive oil adulteration with soft refined oils: preliminary findings from two different laboratories

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SUPPORTING INFORMATION

SECTION 1 – MULTIVARIATE STUDIES

The Total Ion Chromatograms in ESI + ionization of a pure EVOO sample, of mixture A and of mixture H are presented in the figure below.

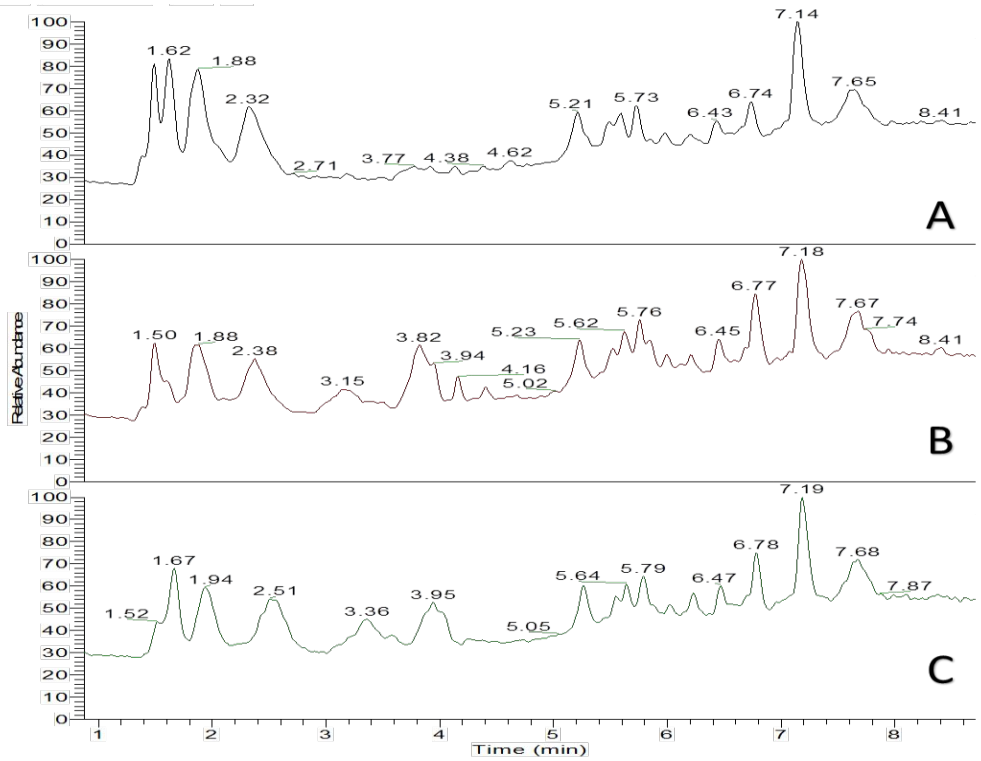


Figure S1. Total Ion Chromatograms (ESI + ionization from 1 to 9 min) of (A) pure EVOO sample; (B) mixture A; (C) mixture H

The PCA scores plot of the results obtained for both the ionization modes including the QC samples are presented in figures S2 and S3.

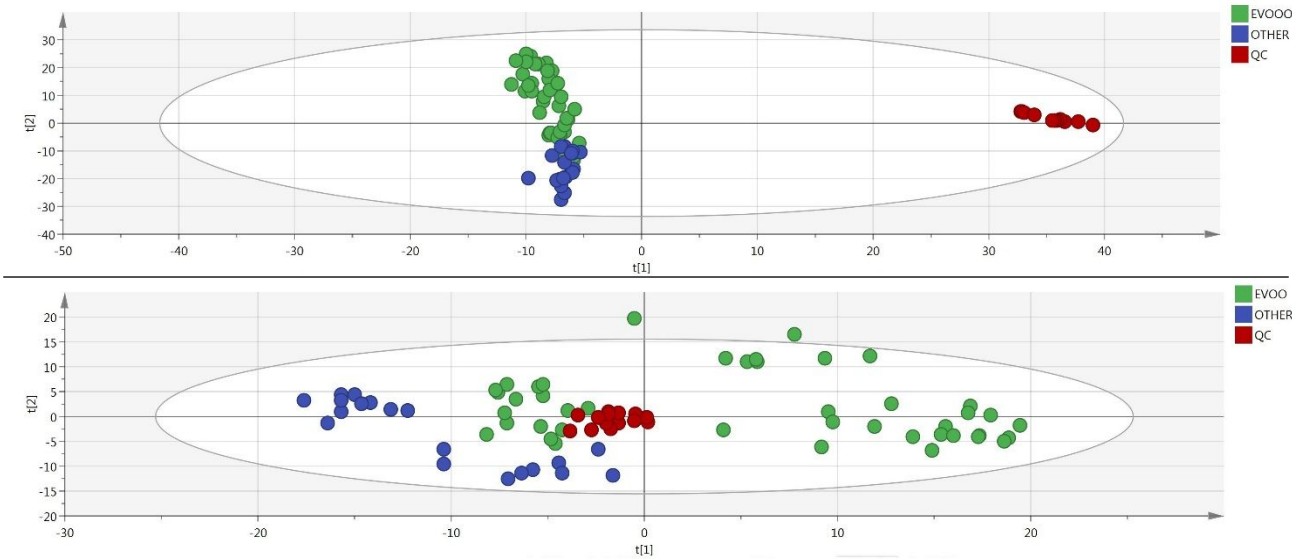


Figure S2. ESI + PCA Scores plots of the samples including QCs (X axis: PC1, Y axis: PC2). A: *Laboratory #1*; number of PCs: 9; explained variance of of each PC: PC1 33.7%, PC2 22.0%, PC3 8.7%, PC4 5.8%, PC5 4.7%, PC6 4.1%, PC7 2.6%, PC8 2.0%, PC9 1.5%,. B: *Laboratory #2; n*umber of PCs: 10; explained variance of each PC: PC1 18.8%, PC2 7.1%, PC3 5.9%, PC4 4.9%, PC5 3.8%, PC6 3.4%, PC7 3.0%, PC8 2.7%, PC9 2.5%, PC10 2.1%. Green dots: "EVOO" samples; blue dots: "OTHER" samples; red dots. "QC" samples.

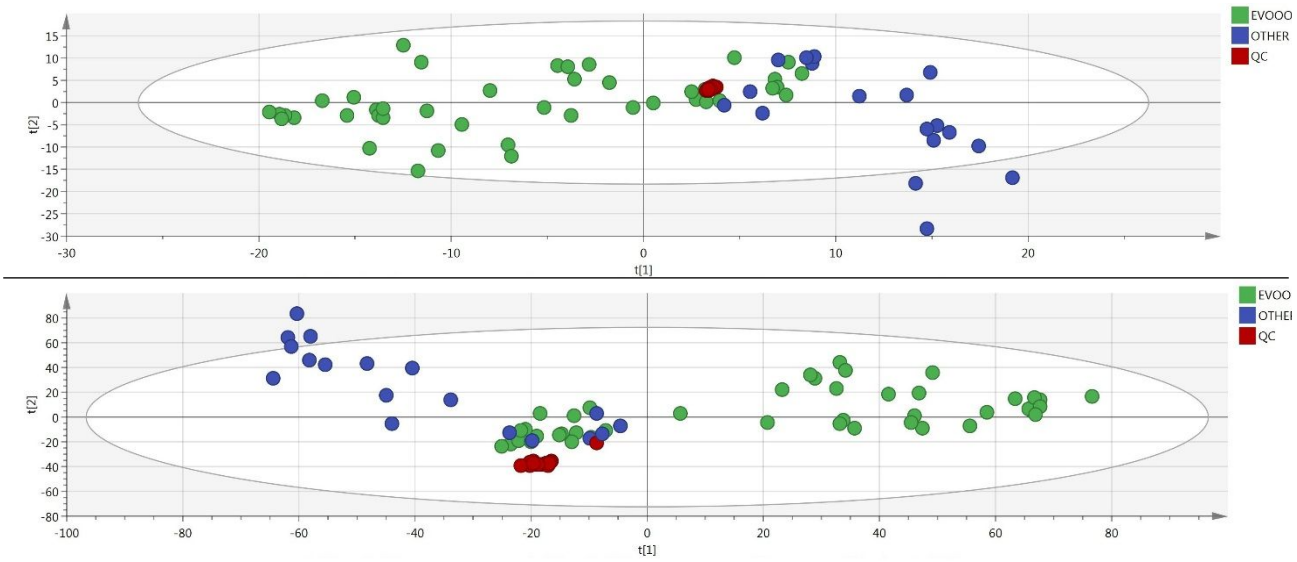


Figure S3. ESI - PCA Scores plots of the samples including QCs (X axis: PC1, Y axis: PC2). A: *Laboratory #1*; number of PCs: 14; explained variance of each PC: PC1 32.7%, PC2 15.9%, PC3 11.1%, PC4 6.9%, PC5 5.9%, PC6 3.4%, PC7 2.9%, PC8 2.4%, PC9 2.1%, PC10 1.8%, PC11 1.3%, PC12 1.2%, PC13 1.0%, PC14 1.0%.:B: *Laboratory #2; n*umber of PCs: 5; explained variance of each PC: PC1 21.2%, PC2 9.5%, PC3 8.2%, PC4 6.4%, PC5 5.1%,. Green dots: "EVOO" samples; blue dots: "OTHER" samples; red dots. "QC" samples.

The PCA and PLS-DA scores plot of the results obtained with the negative ionization mode are presented in figures S4 and S5.

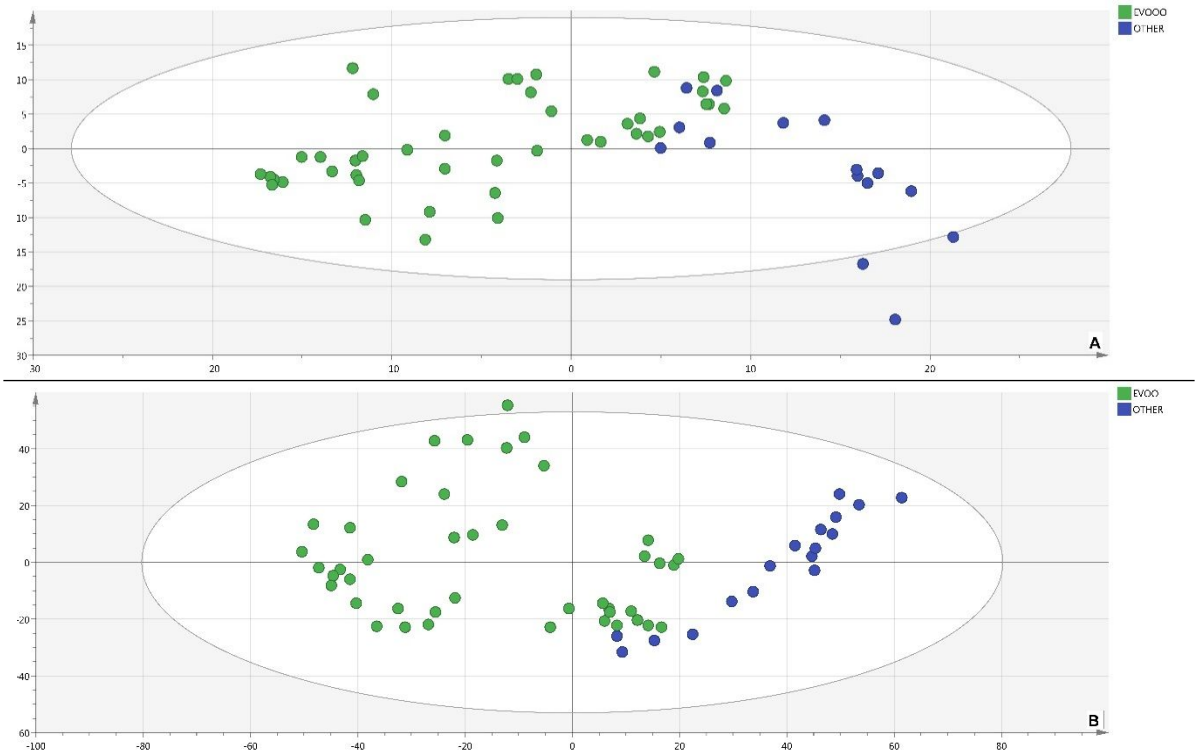


Figure S4. ESI - PCA Scores plots of the samples (X axis: PC1, Y axis: PC2). A: *Laboratory #1*; number of PCs: 9; explained variance of each PC: PC1 33.7%, PC2 15.7%, PC3 11.3%, PC4 7.2%, PC5 5.4%, PC6 3.6%, PC7 3.1%, PC8 2.4%, PC9 2.1%. B: *Laboratory #2; n*umber of PCs: 7; explained variance of each PC: PC1 12.0%, PC2 10.0%, PC3 9.1%, PC4 8.5%, PC5 7.9%, PC6 7.1%, PC7 6.4%. Green dots: "EVOO" samples; blue dots: "OTHER" samples.

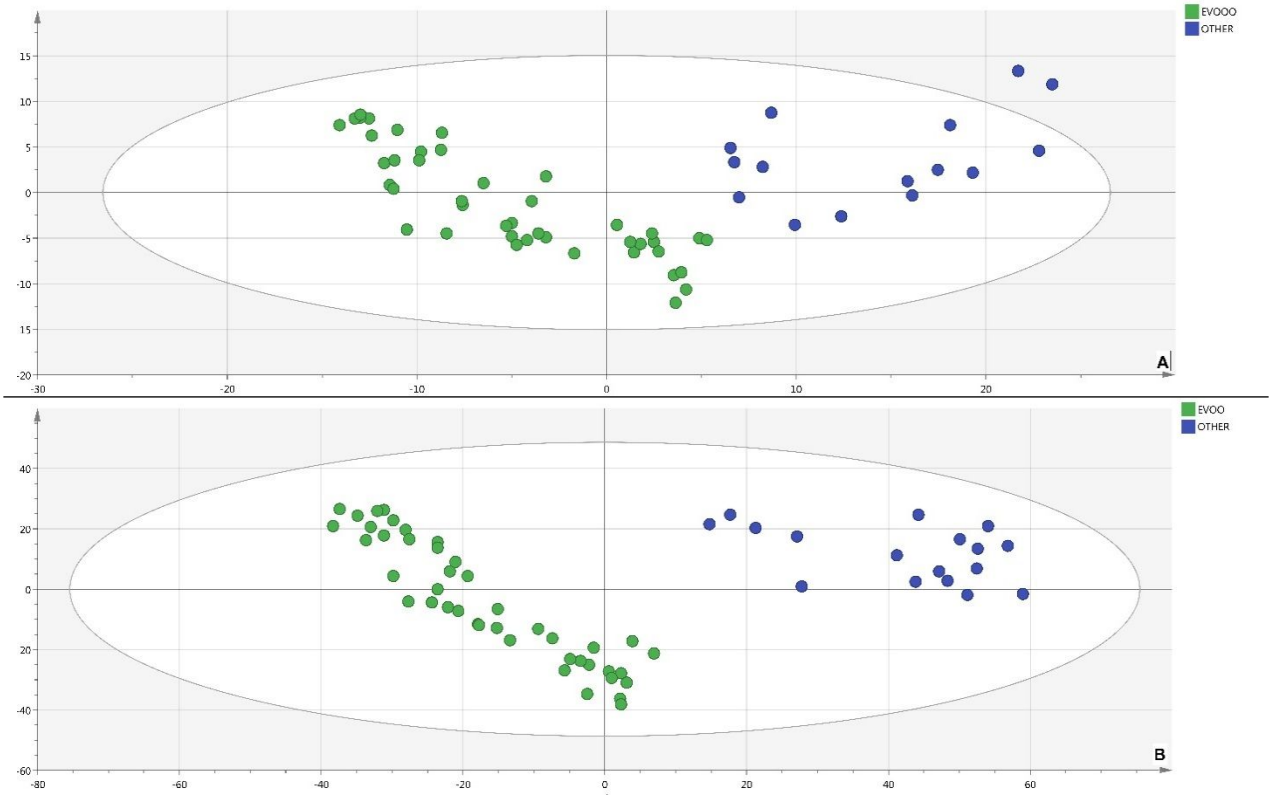


Figure S5. ESI - PLS-DA Scores plots of the samples(X axis: Component 1, Y axis: Component 2). A: *Laboratory #1*; R^2X (cum) = 0.682; R^2Y (cum) = 0.954; Q^2 (cum) = 0.871. B: *Laboratory #2*; R^2X (cum) = 0.235; R^2Y (cum) = 0.989; Q^2 (cum) = 0.906. Green dots: "EVOO" samples; blue dots: "OTHER" samples.

		Laboratory #1		Laboratory #2	
SAMPLE	**CLASS**	**% affinity "EVOO" group**	**% affinity "NOT EVOO" group**	**% affinity "EVOO" group**	**% affinity "NOT EVOO" group**
CP_30	EVOO	88%	12%	91%	9%
CP_31	EVOO	100%	0%	92%	8%
CP_32	EVOO	100%	0%	98%	2%
DEO3	NOT EVOO	0%	100%	22%	78%
DEO_DEA2	NOT EVOO	0%	100%	0%	100%
MIX_D	NOT EVOO	3%	97%	19%	81%

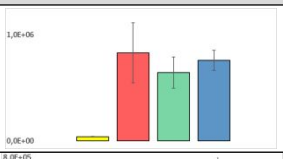
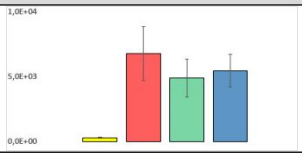
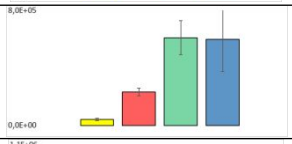
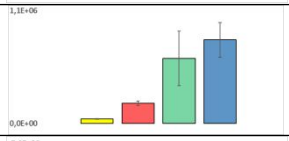
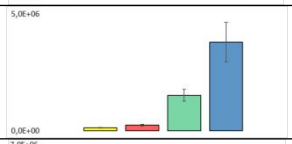
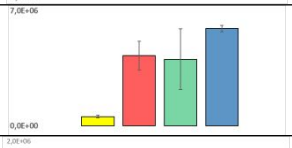
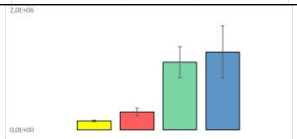
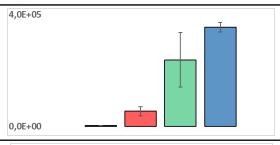
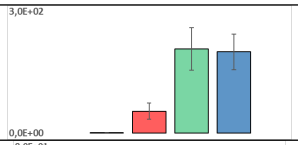
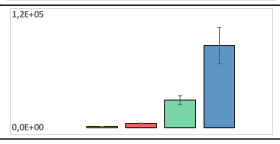
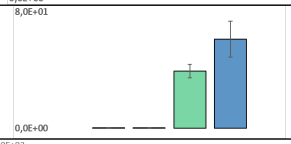
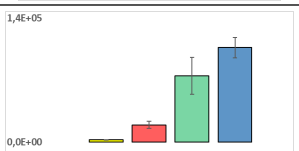
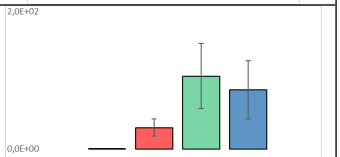
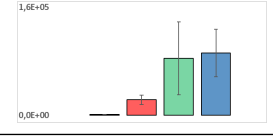
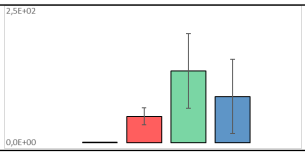
Table *S1*: Predicted group affinity for the samples of the validation set obtained with the ESI+ PLS-DA models. According to the software rules, values higher than 65% correspond to a certain belonging of the sample to that group

		Laboratory #1		Laboratory #2	
SAMPLE	**CLASS**	**% affinity "EVOO" group**	**% affinity "NOT EVOO" group**	**% affinity "EVOO" group**	**% affinity "NOT EVOO" group**
CP_30	EVOO	100%	0%	96%	4%
CP_31	EVOO	100%	0%	100%	0%
CP_32	EVOO	100%	0%	100%	0%
DEO3	NOT EVOO	0%	100%	0%	100%
DEO_DEA2	NOT EVOO	14%	86%	0%	100%
MIX_D	NOT EVOO	12%	88%	0%	66%

Table *S2*: Predicted group affinity for the samples of the validation set obtained with the ESI- PLS-DA models. According to the software rules, values higher than 65% correspond to a certain belonging of the sample to that group

SECTION 2 – COMPOUND IDENTIFICATION

The complete list of the supposed names of the 12 selected compounds is presented in Table S3, together with their identification level [1] and their mean area values in the different groups. The "Variables trends plot" of "Compound 1" and of "Compound 7" are presented in figure S6.

Compound ID	Name	ID Level	*Lab#1* Area values	*Lab#2* Area values
1	Propylene glycol - 1 Stearate	3		
2	4-Phenylbutyric acid	2		N.D.
3	Tyrosine ethyl ester	3		N.D.
4	Geranic Acid	3		N.D.
5	3,4,5-trimethoxydihydrocinnamic acid	3		N.D.
6	Propyl-12-hydroxy-9-octadecenoate	3		N.D.
7	N.A.	4		
8	N.A.	4		
9	(2R,3E)-5-(3-Chloro-5-formyl-2,6-dihydroxy-4-methylphenyl)-3-methyl-1-[(1S,2R,6R)-1,2,6-trimethyl-3-oxocyclohexyl]-3-penten-2-yl acetate	2		
10	N.A.	4		

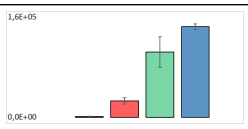
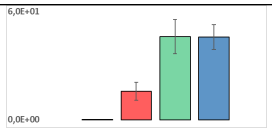
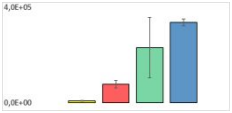
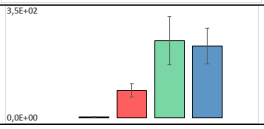
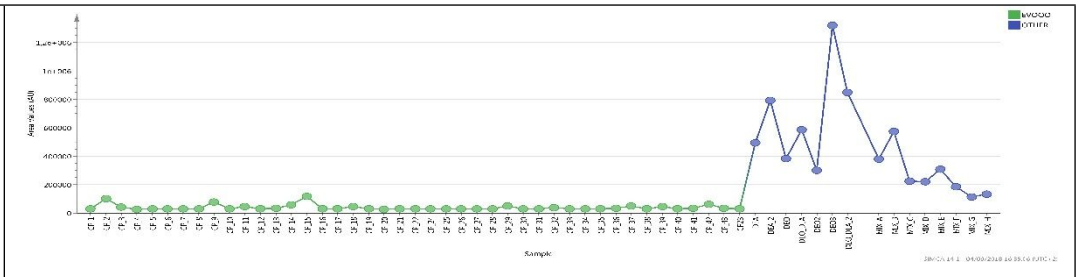
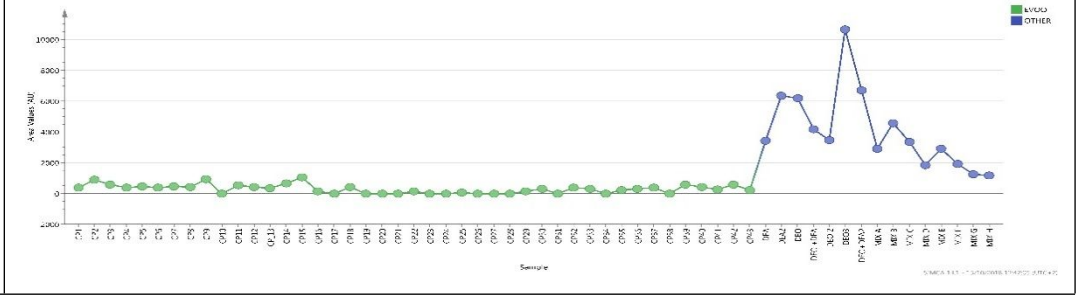
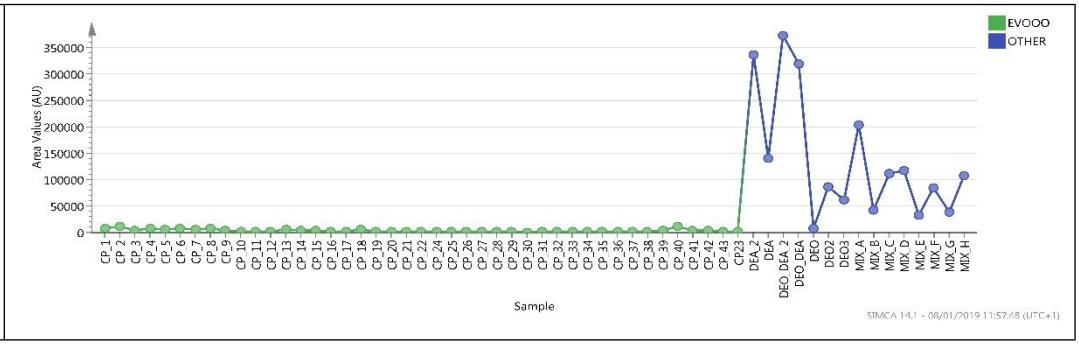
11	N.A.	4		
12	2,3,4-Trihydroxy-6-methyl-5-[(2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-yl]benzaldehyde	3		

Table *S3*: compounds description and comparison of their mean area values (+/− standard error) through the groups (yellow bar: EVOO; red bar: DEO; green bar: DEA, blue bar: DEO+DEA samples) N.A.= Not available

COMPOUND 1

LABORATORY #1	
LABORATORY #2	

COMPOUND 7

LABORATORY #1	

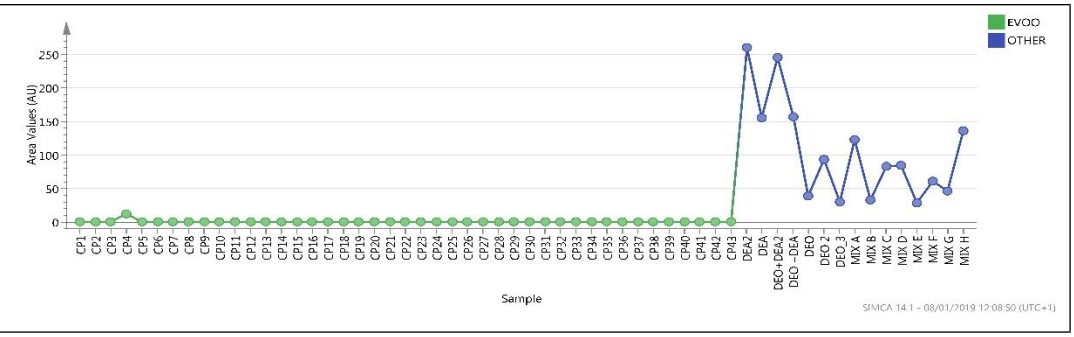
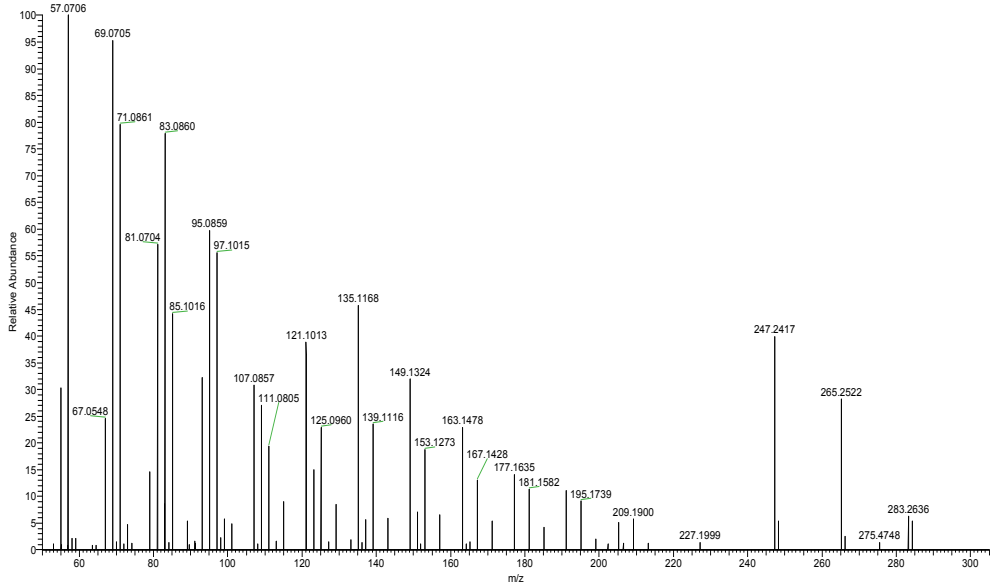
LABORATORY #2	

Figure S6. Variable trend plots of "Compound 1" and of "Compound 7". Green dots: area values of the marker in "EVOO" group; blue dots: area values of the marker in "OTHER" group.

SECTION 3 – EVALUATION OF THE INTER-LABORATORY STUDY

The comparison of the MS/MS spectra of the compounds selectedwith the two instrument is presented in the following figures

LABORATORY #1	

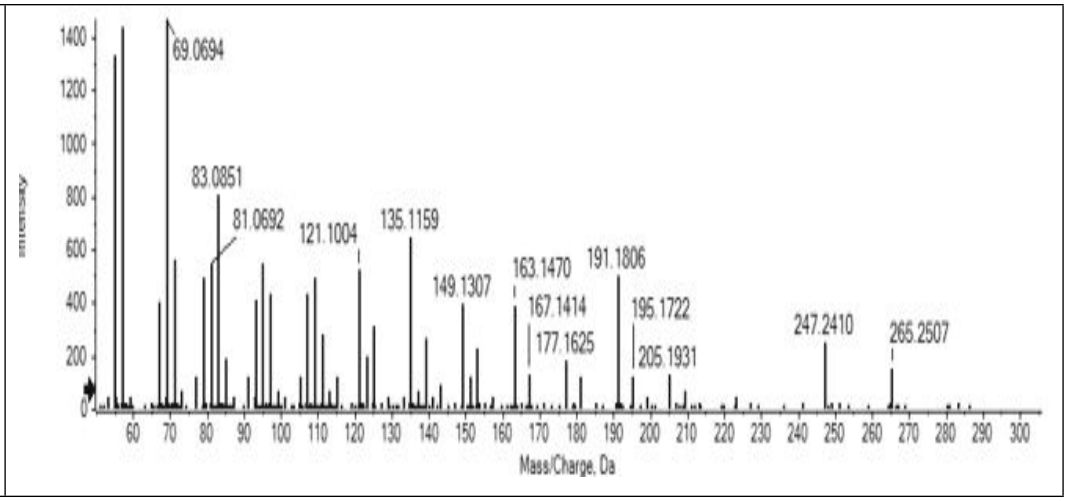
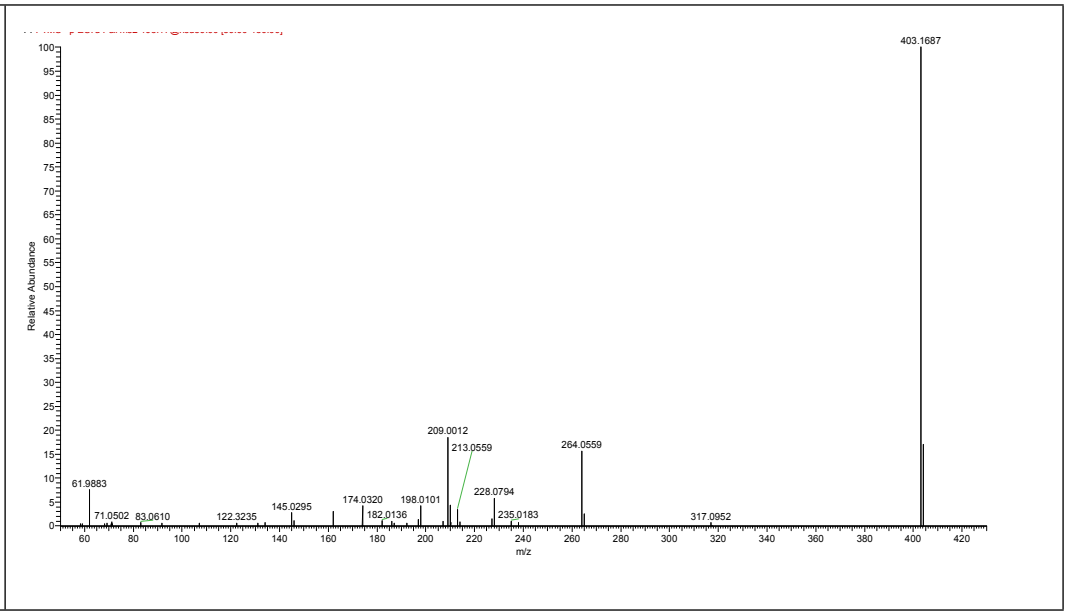
LABORATORY #2	

Figure S7 Comparison of the MS/MS spectra of the "Compound 1" obtained in *Laboratory #1* and in *Laboratory #2*. Instrumental conditions are detailed in the text

LABORATORY #1	

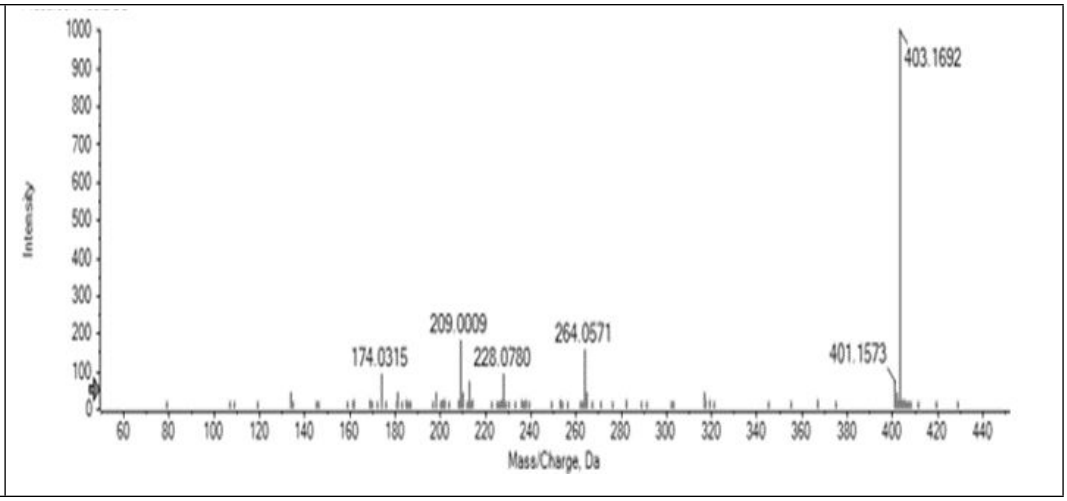
LABORATORY #2	

Figure S8 Comparison of the MS/MS spectra of the "Compound 7" obtained in *Laboratory #1* and in *Laboratory #2*. Instrumental conditions are detailed in the text

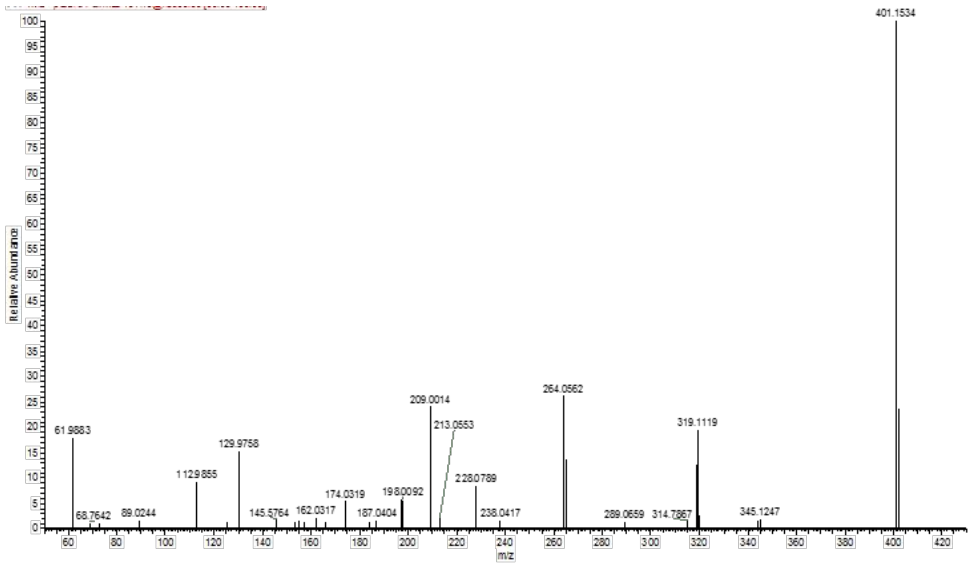
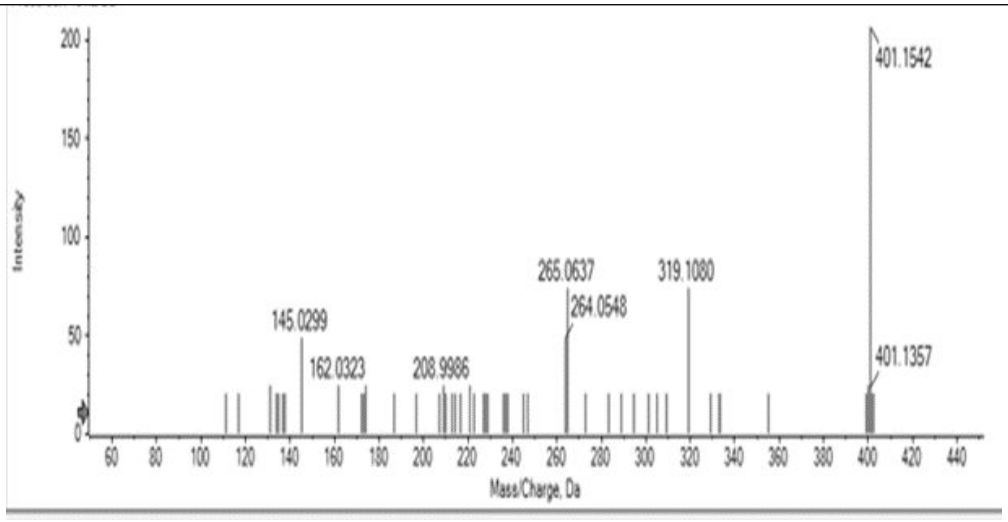
LABORATORY #1	
LABORATORY #2	

Figure S9 Comparison of the MS/MS spectra of the "Compound 8" obtained in *Laboratory #1* and in *Laboratory #2*. Instrumental conditions are detailed in the text

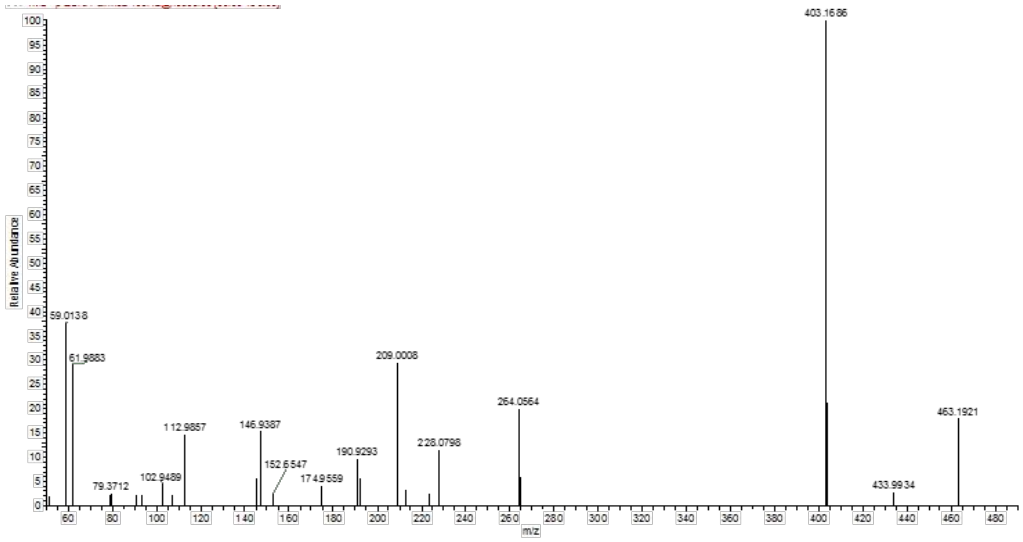
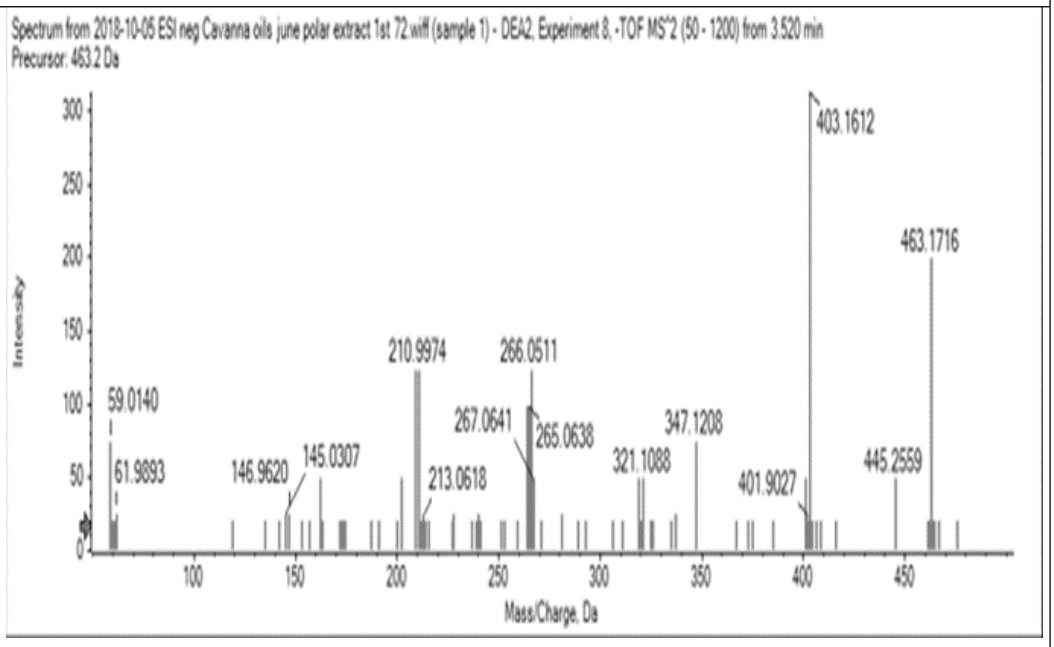
LABORATORY #1	
LABORATORY #2	

Figure S10 Comparison of the MS/MS spectra of the "Compound 9" obtained in *Laboratory #1* and in *Laboratory #2*. Instrumental conditions are detailed in the text

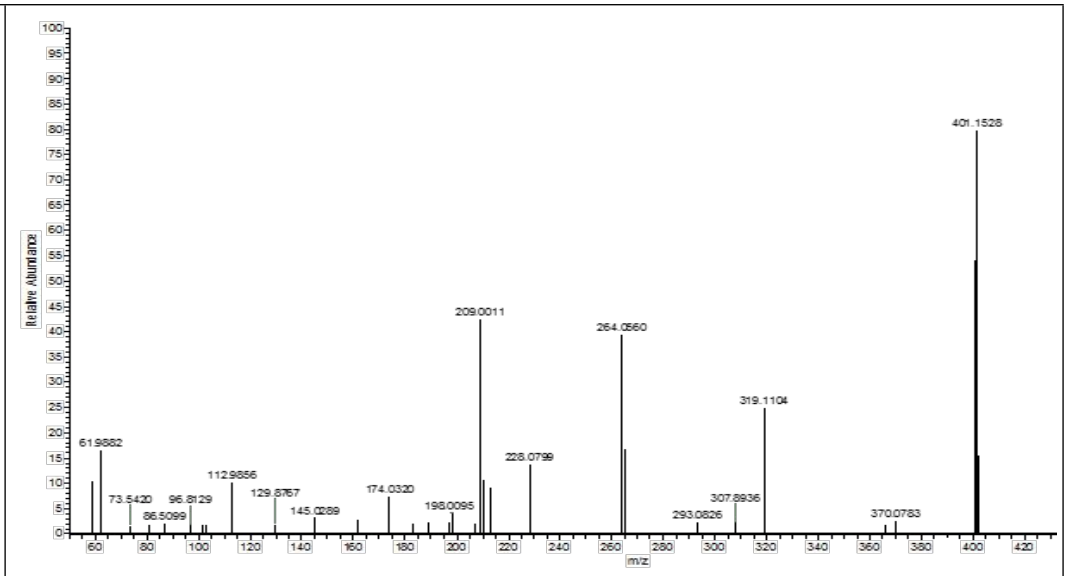
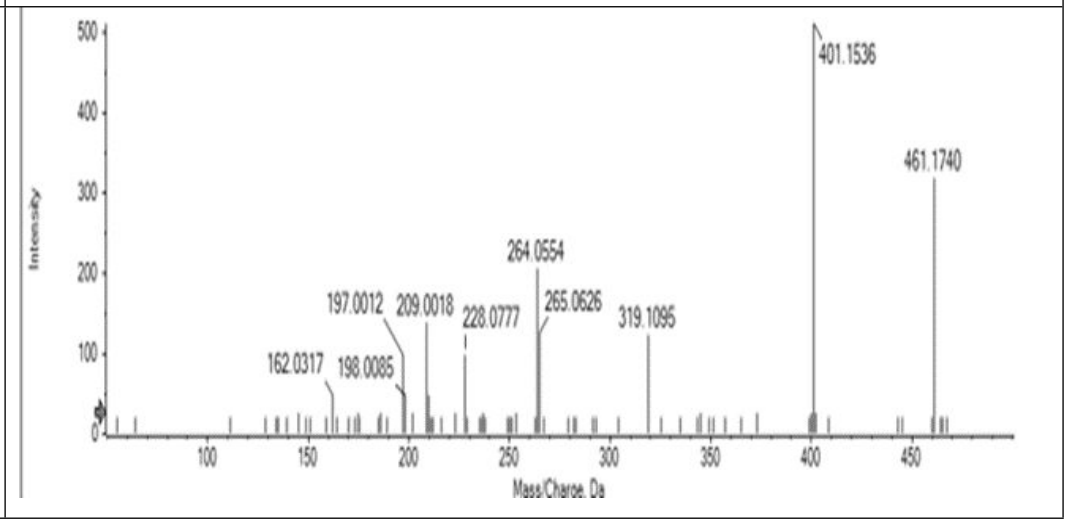
LABORATORY #1	
LABORATORY #2	

Figure S11 Comparison of the MS/MS spectra of the "Compound 10" obtained in *Laboratory #1* and in *Laboratory #2*. Instrumental conditions are detailed in the text

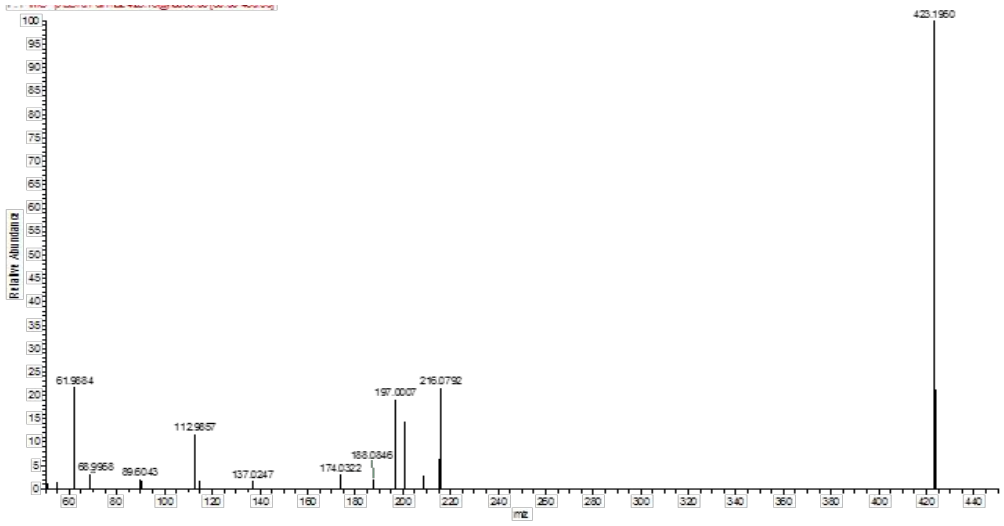
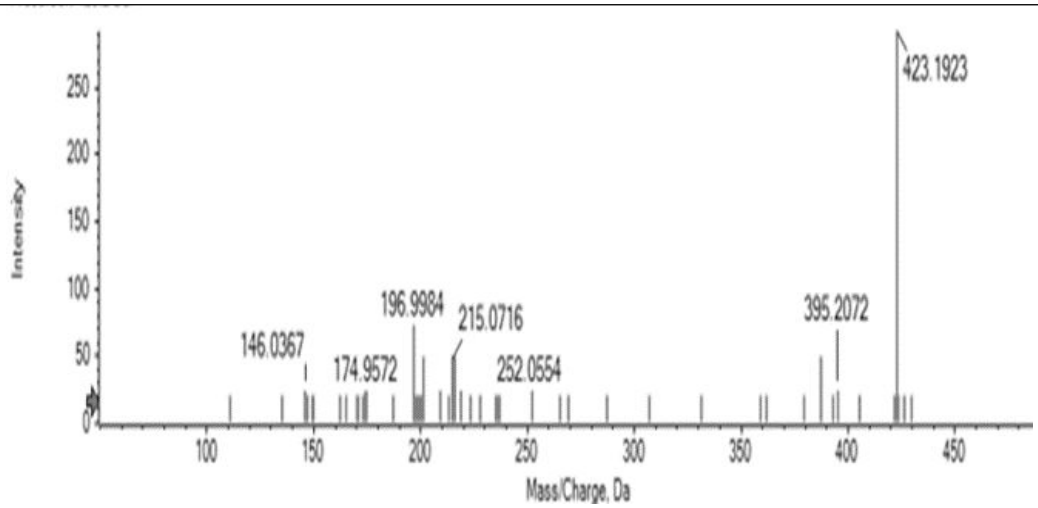
LABORATORY #1	
LABORATORY #2	

Figure S12 Comparison of the MS/MS spectra of the "Compound 11" obtained in *Laboratory #1* and in *Laboratory #2*. Instrumental conditions are detailed in the text

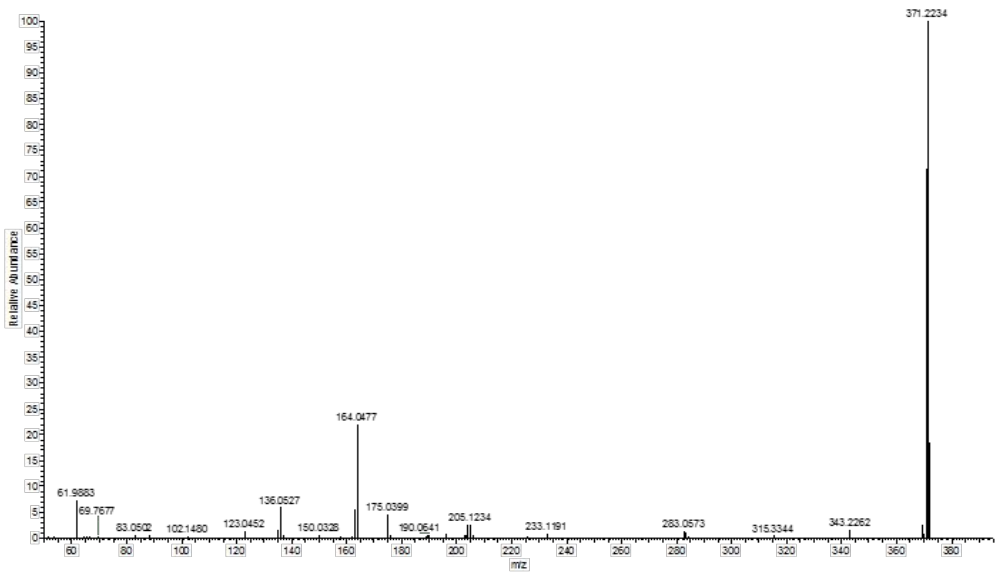
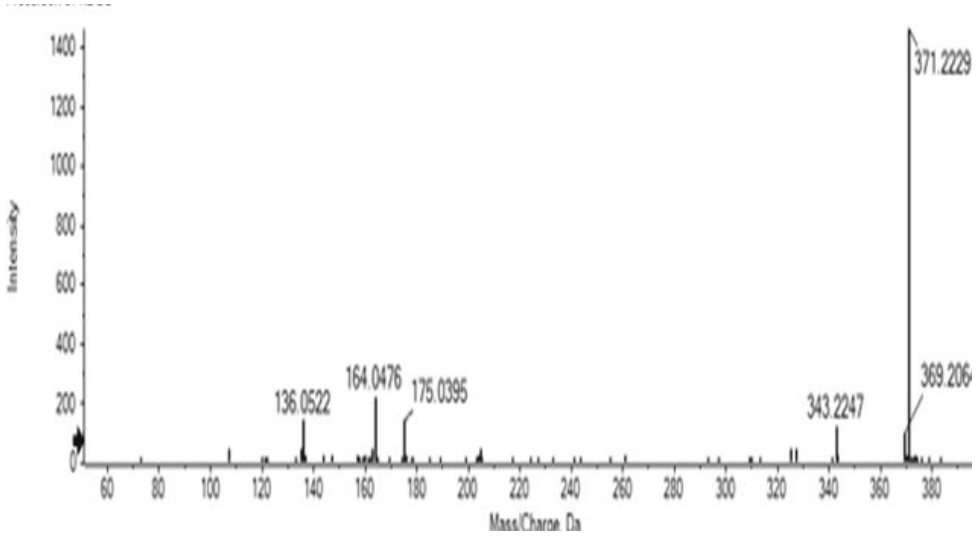
LABORATORY #1	
LABORATORY #2	

Figure S13 Comparison of the MS/MS spectra of the "Compound 12" obtained in *Laboratory #1* and in *Laboratory #2*. Instrumental conditions are detailed in the text

SECTION 4 – SAMPLING PLAN

The global list of the samples used in this study is detailed in table S4.

NAME	TYPE	YEAR	SUPPLIER	STORAGE
CP_1	EVOO	2015/2016	1	2-8°C
CP_2	EVOO	2015/2016	1	2-8°C
CP_3	EVOO	2015/2016	2	2-8°C
CP_4	EVOO	2015/2016	2	2-8°C
CP_5	EVOO	2015/2016	2	2-8°C
CP_6	EVOO	2015/2016	2	2-8°C
CP_7	EVOO	2015/2016	2	2-8°C
CP_8	EVOO	2015/2016	2	2-8°C
CP_9	EVOO	2015/2016	1	2-8°C
CP_10	EVOO	2015/2016	3	2-8°C
CP_11	EVOO	2015/2016	3	2-8°C
CP_12	EVOO	2015/2016	3	2-8°C
CP_13	EVOO	2016/2017	2	2-8°C
CP_14	EVOO	2016/2017	2	2-8°C
CP_15	EVOO	2016/2017	2	2-8°C
CP_16	EVOO	2016/2017	2	2-8°C
CP_17	EVOO	2016/2017	2	2-8°C
CP_18	EVOO	2016/2017	2	2-8°C
CP_19	EVOO	2016/2017	2	2-8°C
CP_20	EVOO	2017/2018	2	2-8°C
CP_21	EVOO	2017/2018	2	2-8°C
CP_22	EVOO	2017/2018	2	2-8°C
CP_23	EVOO	2017/2018	2	2-8°C
CP_24	EVOO	2017/2018	2	2-8°C
CP_25	EVOO	2017/2018	2	2-8°C
CP_26	EVOO	2017/2018	2	2-8°C
CP_27	EVOO	2017/2018	2	2-8°C
CP_28	EVOO	2017/2018	2	2-8°C
CP_29	EVOO	2017/2018	2	2-8°C
CP_30	EVOO	2017/2018	2	2-8°C
CP_31	EVOO	2017/2018	2	2-8°C
CP_32	EVOO	2016/2017	2	2-8°C
CP_33	EVOO	2015/2016	2	2-8°C
CP_34	EVOO	2017/2018	2	2-8°C
CP_35	EVOO	2017/2018	2	2-8°C
CP_36	EVOO	2017/2018	2	2-8°C
CP_37	EVOO	2017/2018	2	2-8°C
CP_38	EVOO	2017/2018	2	2-8°C
CP_39	EVOO	2015/2016	1	Room Temperature
CP_40	EVOO	2015/2016	1	Room Temperature
CP_41	EVOO	2015/2016	1	Room Temperature

NAME	TYPE	YEAR	SUPPLIER	STORAGE
CP_42	EVOO	2015/2016	1	Room Temperature
CP_43	EVOO	2015/2016	1	Room Temperature
DEO	DEODORIZED	/	/	2-8°C
DEO2	DEODORIZED	/	/	2-8°C
DEO3	DEODORIZED	/	/	2-8°C
DEA	DEACIDIFIED	/	/	2-8°C
DEA2	DEACIDIFIED	/	/	2-8°C
DEO_DEA	DEODORIZED AND DEACIDIFIED	/	/	2-8°C
DEO_DEA2	DEODORIZED AND DEACIDIFIED	/	/	2-8°C
MIX A	EVOO 25% + DEA-DEO 75%	/	/	2-8°C
MIX B	EVOO 25% + DEO 75%	/	/	2-8°C
MIX C	EVOO 55% + DEA-DEO 45%	/	/	2-8°C
MIX D	EVOO 60%+ DEA-DEO 40%	/	/	2-8°C
MIX E	EVOO 50% + DEO 50%	/	/	2-8°C
MIX F	EVOO 40% + DEO 60%	/	/	2-8°C
MIX G	Commercial DEO sample	/	/	2-8°C
MIX H	Commercial DEO sample	/	/	2-8°C

Table S*4*: list and description of the samples. CP_19, CP_26 and CP_31 were produced with olives coming from European Union

REFERENCES

[1] Schymanski, E.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H.; Hollender, J. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environ. Sci. Technol.* **2014***,* 48, 2097-2098.