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GRASSE

A harmonized multi-analyte SPME GC-FID or GC-MS method for measuring volatile compounds in virgin olive oil: some evidence from the validation process

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Perugia, June 16, 2022

Volatile compounds

Premise

- ✓ The volatile compounds, as molecules strongly linked with olive oils sensory profiles, should be considered a relevant quality markers for OOs.

SCAN ME

Trends in Food Science & Technology 105 (2020) 483–493

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Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

ELSEVIER

Review

Olive oil quality and authenticity: A review of current EU legislation, standards, relevant methods of analyses, their drawbacks and recommendations for the future

Lanfranco Conte[a], Alessandra Bendini[b,*], Enrico Valli[b], Paolo Lucci[a], Sabrina Moret[a], Alain Maquet[c], Florence Lacoste[d], Paul Brereton[e], Diego Luis García-González[f], Wenceslao Moreda[f], Tullia Gallina Toschi[b]

- ✓ The determination of these compounds could support the sensory analysis, especially within the so-called “boundary zones”.
- ✓ During the last years researchers are working hard for the setting up of robust analytical methods for evaluating the quali-quantitative profiles of volatile compounds in OOs.
- ✓ Further research efforts should be done in focusing on a low number of volatile compounds, previously selected as relevant markers of the sensory defects, to be determined by possibly using less expensive instruments, such as SPME-GC-FID.

Oleum

Method for the assessment of the organoleptic characteristics (**Quantitative Panel Test**)

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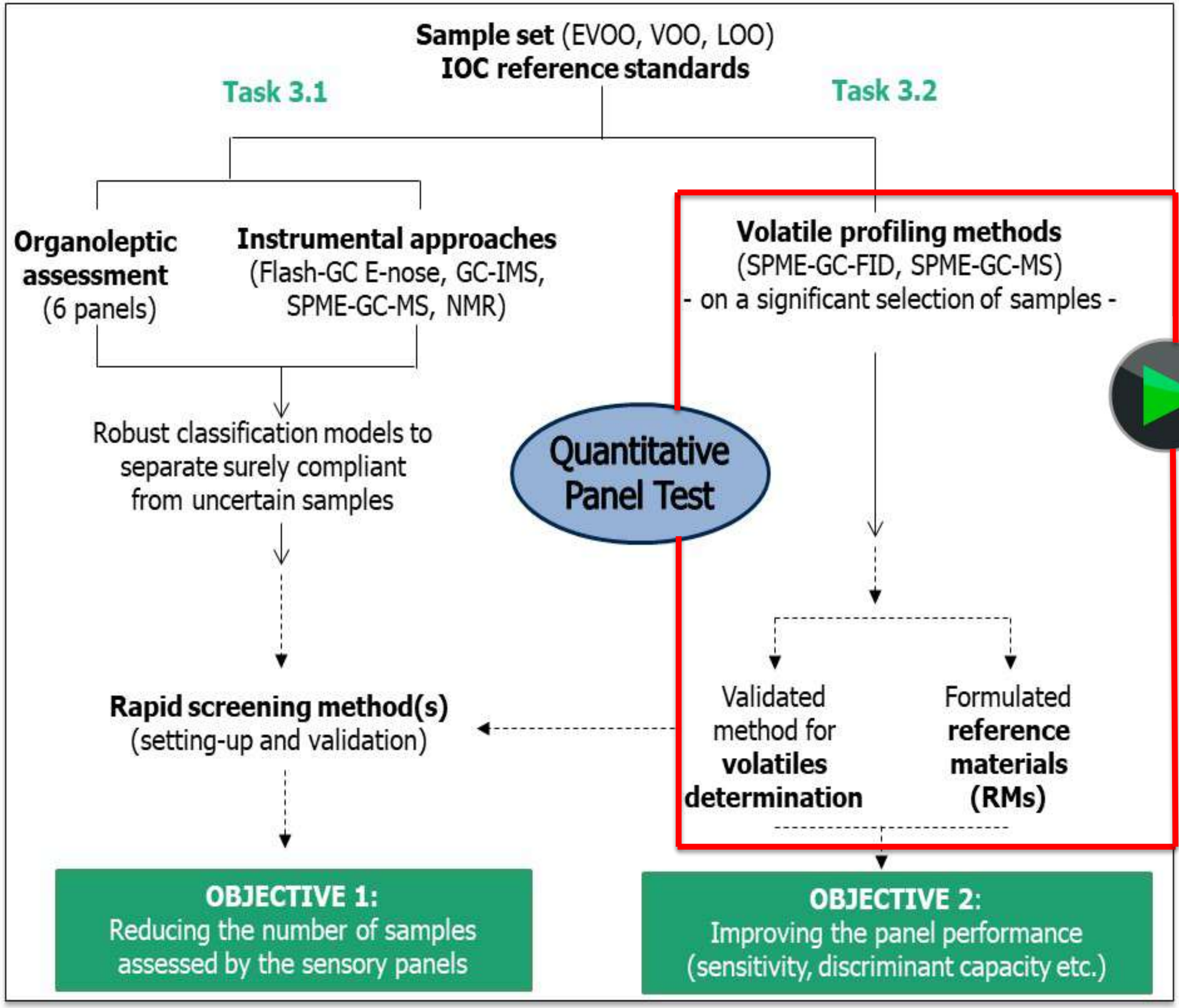

Sample set (EVOO, VOO, LOO)
IOC reference standards

Task 3.1

Task 3.2

Organoleptic assessment
(6 panels)

Instrumental approaches
(Flash-GC E-nose, GC-IMS, SPME-GC-MS, NMR)

Robust classification models to separate surely compliant from uncertain samples

Rapid screening method(s)
(setting-up and validation)

Quantitative Panel Test

Volatile profiling methods
(SPME-GC-FID, SPME-GC-MS)
- on a significant selection of samples -

Validated method for **volatiles determination**

Formulated **reference materials (RMs)**

OBJECTIVE 1:
Reducing the number of samples assessed by the sensory panels

OBJECTIVE 2:
Improving the panel performance (sensitivity, discriminant capacity etc.)

Starting point

SPME-GC-MS/FID
Targeted methods

All **this information could be useful** to confirm or disconfirm the quality grade classification made by panel test, **in case of disagreement between panels.**

OLEUM Developers of the methods

SPME-GC-FID

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INSTITUTO DE LA GRASA CSIC

Standardized procedure

7 labs applying the same method on the same samples, analyzed by 6 sensory panels.

SPME-GC-MS

STUDIUM GENERALE CIVITATIS PERUSII A.D. MCCCVIII

ITERG Expertise Corps Gras

Nestlé Research

UNIVERSITAS STUDIORUM UTINENSIS

UNIVERSITAT DE BARCELONA

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IDENTIFICATION OF SOURCE OF ERRORS

A standardize procedure

Error in the analysis = Adding compounds + Identification + ✗ Instrumentation (e.g. short columns) + Wrong integration + Quantitation procedure

Check/revise data chromatograms

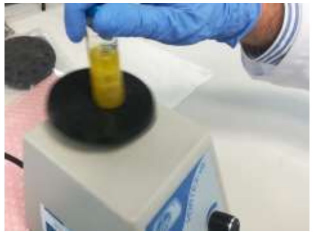
A single procedure

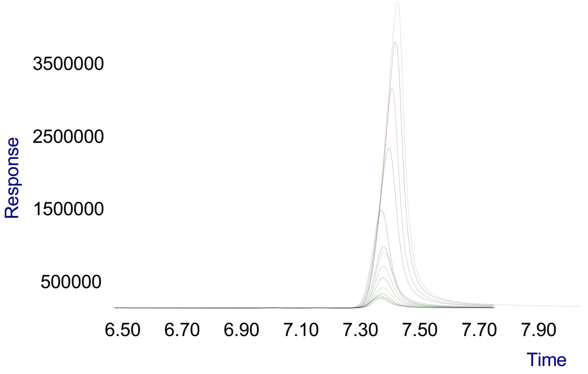
Harmonized method
(variables defined and optimized)

Simplicity
(avoid time consuming and confussing procedures)

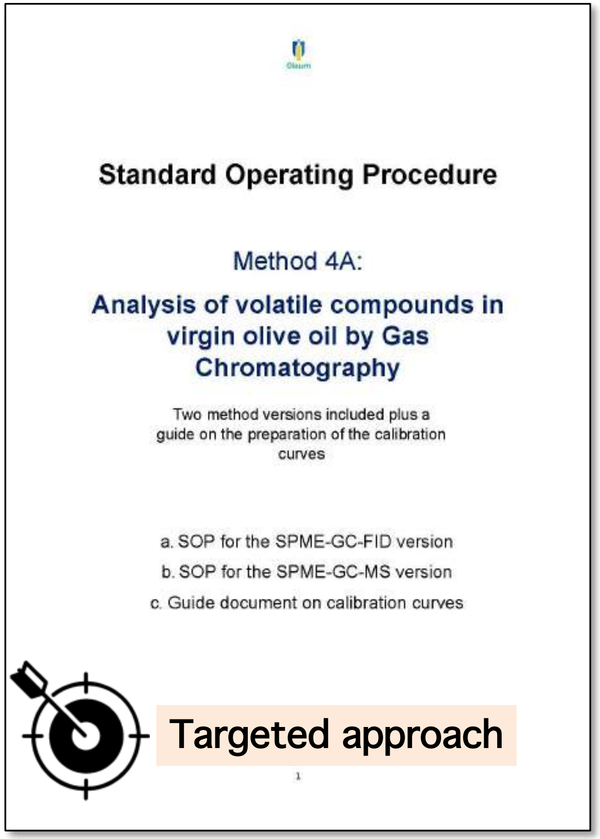
Error reduction
(e.g. define calibration procedure)

Adaptable to validation
(e.g. quantitative output)





A harmonized multi-analyte SPME GC-FID or GC-MS method for measuring volatile compounds in virgin olive oil



Standard Operating Procedure

Method 4A:

Analysis of volatile compounds in virgin olive oil by Gas Chromatography

Two method versions included plus a guide on the preparation of the calibration curves

a. SOP for the SPME-GC-FID version

b. SOP for the SPME-GC-MS version

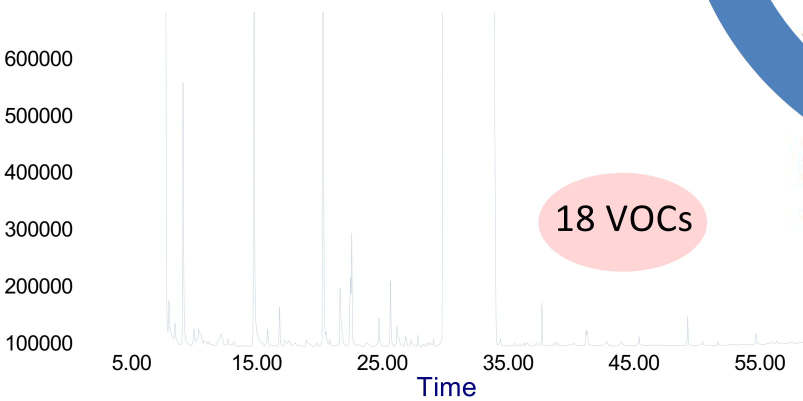
c. Guide document on calibration curves

Targeted approach

a. SOP for the SPME-GC-**FID** version

b. SOP for the SPME-GC-**MS** version

c. Guide document on **calibration curves**



18 VOCs

Scientific community

- Extraction capacity
- Maximum information

Olive oil actors

- Simplicity
- Understandable

Regulation bodies

- Clear definition of purpose
- Internationally accepted

18 selected volatile compounds as the minimum number of sensory markers

Negative attributes (defects)

Fusty/muddy sediment (Total: 5)
Octane
Ethanol
3-methyl-1-butanol
Propanoic acid
6-methyl-5-hepten-2-one

Winey-vinegary (Total: 3)
Acetic acid
Ethyl acetate
Ethanol

Musty-humid-earthy (Total: 3)
(*E*)-2-heptenal
1-octen-3-ol
Propanoic acid

Frostbitten olives (wet wood) (Total: 1)
Ethyl propanoate

Rancid (Total: 5)
Hexanal
Nonanal
(*E,E*)-2,4-hexadienal
(*E*)-2-decenal
Pentanoic acid

Positive attribute (fruity)

Fruity (Total: 3)
(*E*)-2-hexenal
(*Z*)-3-hexenyl acetate
1-hexanol

1 procedure
2 detectors (FID and MS)

Versatility and adaptability in the implementation of the method.

Measurands: Selected volatile compounds (VOCs) in virgin olive oils (in mg/kg).

Selection criteria: Those VOCs with a demonstrated influence on aroma (sensory defects).

18 VOCs

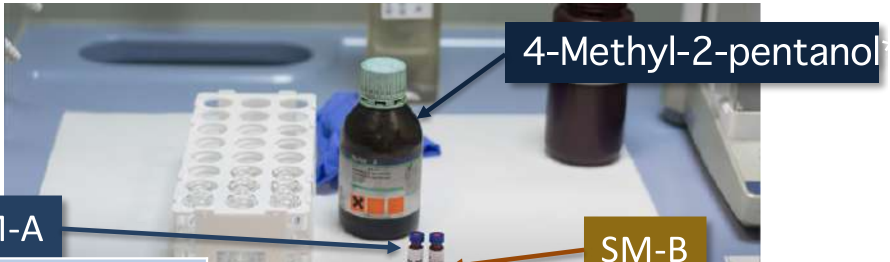
Fermentative defects *(fusty/muddy, winey vinegary, musty)* + Damaged olives + Oxidation *(rancid)* + Positive attributes (fruity)

1. Octane
2. Ethyl acetate
3. Ethanol
4. Ethyl propanoate
5. Hexanal
6. 3-Methyl-1-butanol
7. (*E*)-2-Hexenal
8. (*Z*)-3-Hexenyl acetate
9. (*E*)-2-Heptenal
10. 6-Methyl-5-hepten-2-one
11. 1-Hexanol
12. Nonanal
13. 1-Octen-3-ol
14. (*E,E*)-2,4-Hexadienal
15. Acetic acid
16. Propanoic acid
17. (*E*)-2-Decenal
18. Pentanoic acid

*Internal standard: 4-methyl-2-pentanol

2 Standard mixtures: SM A & SM B

Balance between overlapping at high concentrations, competition phenomena, and concentration ranges.



Low concentration mixture (A) (0.05-10.00 mg/kg)
Octane
Ethyl acetate
Ethyl propanoate
3-Methyl-1-butanol
(*E*)-2-Heptenal
6-Methyl-5-hepten-2-one
(*E,E*)-2,4-hexadienal
Propanoic acid
(*E*)-2-Decenal
Pentanoic acid

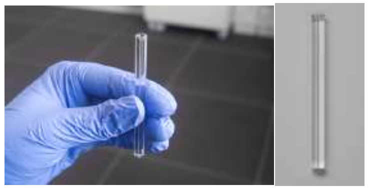
High concentration mixture (B) (0.20-25.00 mg/kg)
Ethanol
Hexanal
(*E*)-2-Hexenal
(*Z*)-3-Hexenyl acetate
1-Hexanol
Nonanal
1-Octen-3-ol
Acetic acid

Apparatus

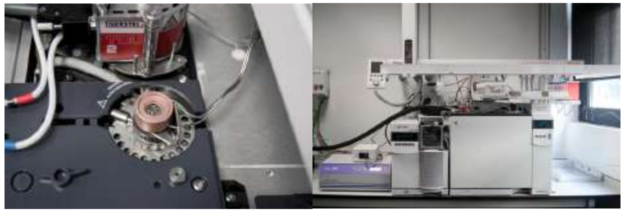
Headspace glass vial, 20 mL.

Septum and aluminium seals

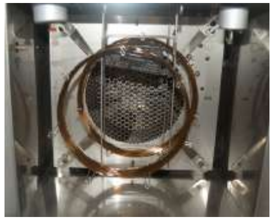
SPME-Liner



Gas chromatograph equipped with a **split-splitless injector** and a **FID/MS detector**.



Capillary column, fused silica, a polar phase based on polyethylene glycol (**PEG**) (e.g. ZB-WAX or TR-WAX), length 60 m, internal diameter 0.25 mm and coating 0.25–0.50 μm.



SPME fiber, length **1 cm**, 50/30 μm film thickness and it is endowed with the Stable Flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane (**DVB/CAR/PDMS**)

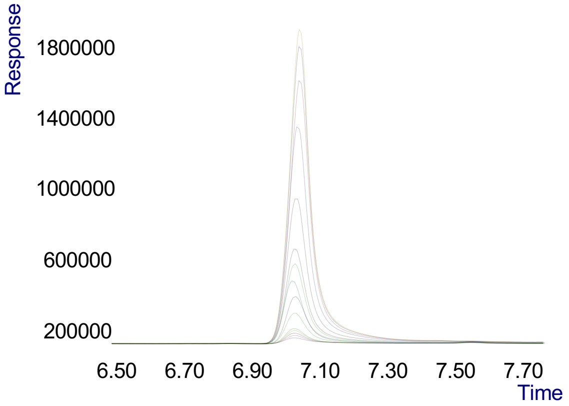
Equilibration time: 10 min at 40 °C under agitation
Fiber exposition: 40 min at 40 °C
Injection port: 5 min, Splitless, 250°C
Carrier gas: He or H_2.
Flow rate: 1.5 mL/min
Temp. Programme: 40°C (10 min), 3°C/min until 200°C (a cleaning step can be added; 20 °C/min to 250 °C for 5 min)

QUANTIFICATION: 2 Standard mixtures - **SM A & SM B**

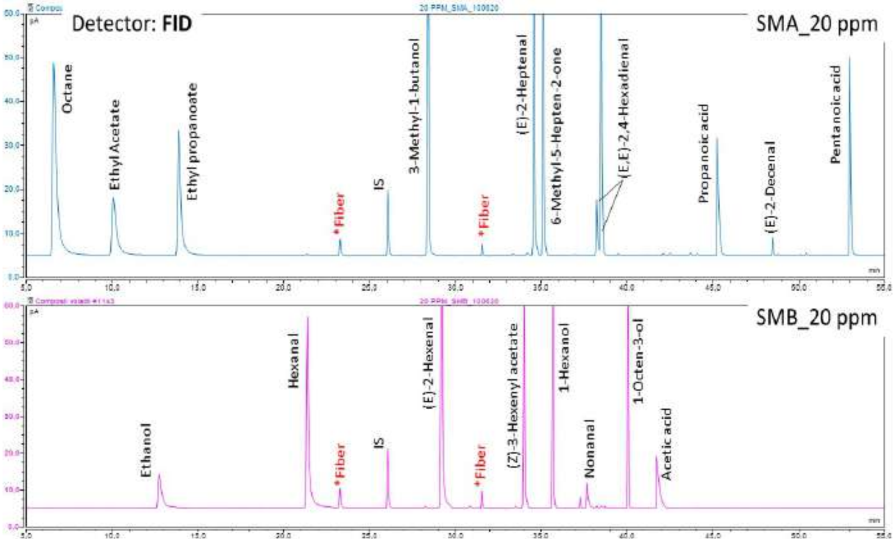
WP3 - Analytical solutions addressing olive oil quality issues

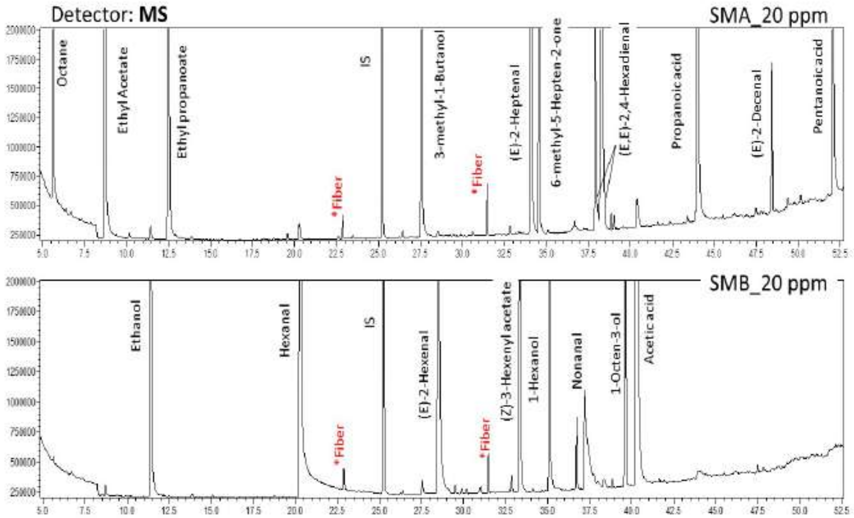
Task 3.2. Protocol for the preparation of sample and calibration curves for volatile analysis.

OBJECTIVE: to avoid the errors coming from the preparation of the calibration curves. To work with exactly the same procedure.

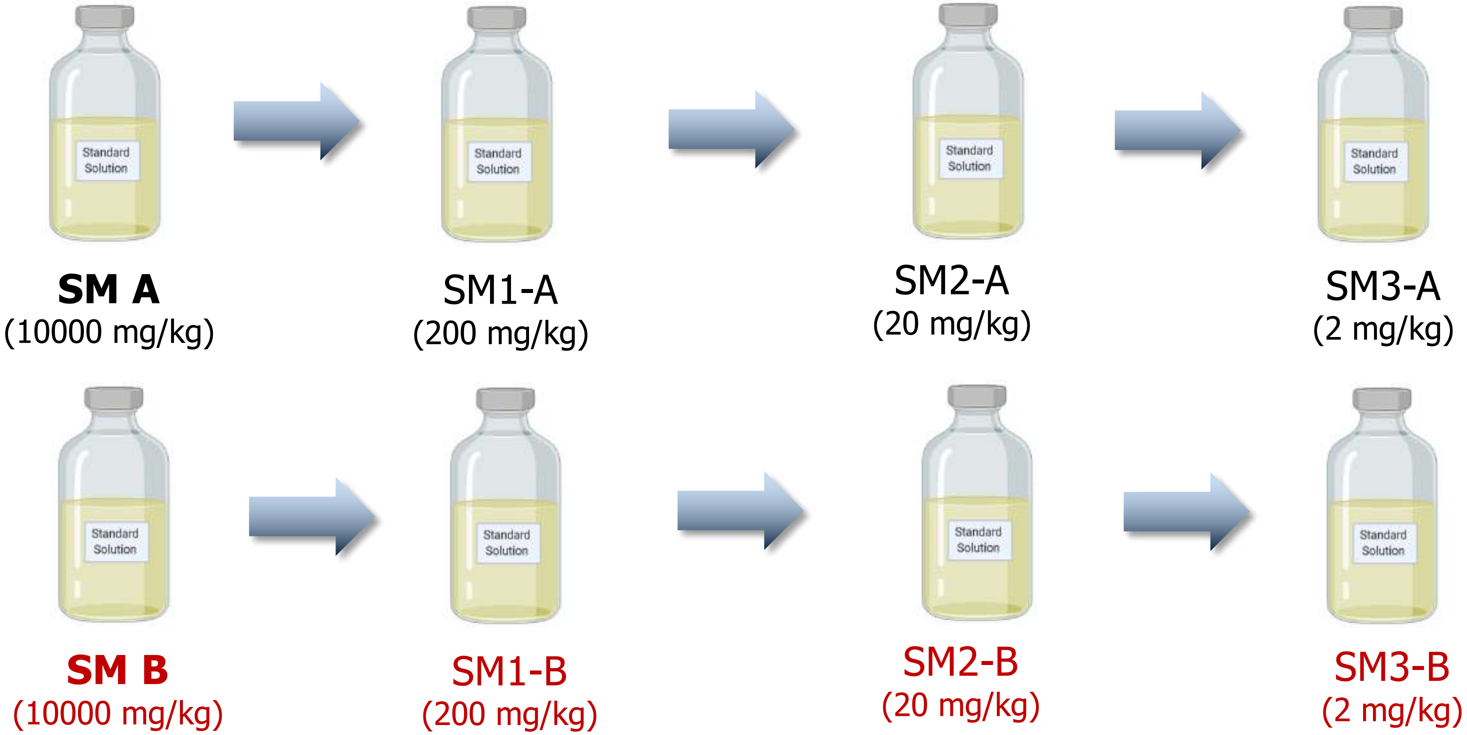


- ✓ An harmonized protocol for building the calibration curves.
- ✓ The exact concentrations needs to be used in all cases.





Preparation of the samples for building the calibration curves



SM A
(10000 mg/kg)

SM1-A
(200 mg/kg)

SM2-A
(20 mg/kg)

SM3-A
(2 mg/kg)

SM B
(10000 mg/kg)

SM1-B
(200 mg/kg)

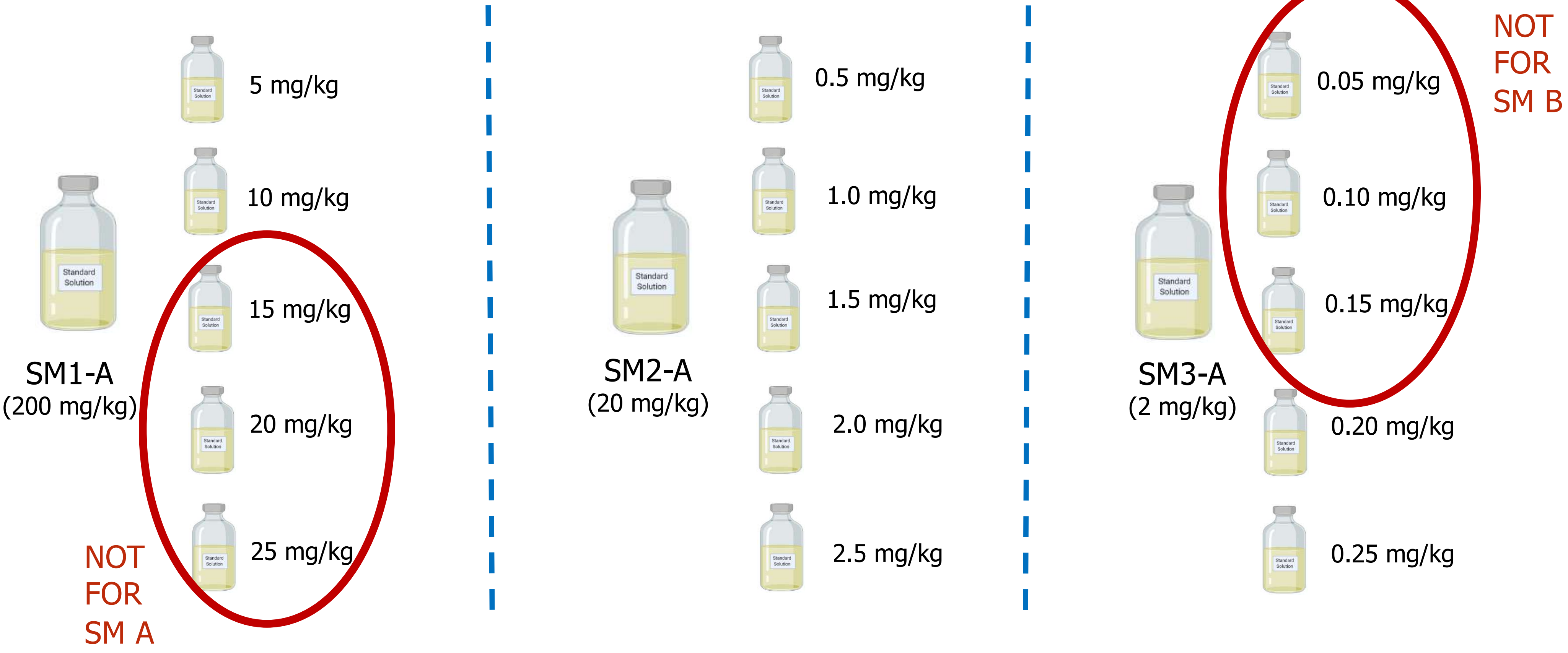
SM2-B
(20 mg/kg)

SM3-B
(2 mg/kg)

Note: Storage conditions and initial steps for the calibration curves preparation.
Do not forget to write down the weights for concentration calculation.
Work at controlled room temperature (T=20-25°C) due to the volatility of the standards.

Preparation of the samples for building the calibration curves

EXAMPLE (SM A)



SM1-A
(200 mg/kg)

5 mg/kg
10 mg/kg
15 mg/kg
20 mg/kg
25 mg/kg

NOT
FOR
SM A

SM2-A
(20 mg/kg)

0.5 mg/kg
1.0 mg/kg
1.5 mg/kg
2.0 mg/kg
2.5 mg/kg

SM3-A
(2 mg/kg)

0.05 mg/kg
0.10 mg/kg
0.15 mg/kg
0.20 mg/kg
0.25 mg/kg

NOT
FOR
SM B

Note: Storage conditions and initial steps for the calibration curves preparation. Do not forget to write down the weights for concentration calculation. Work at controlled room temperature (T=20-25°C) due to the volatility of the standards. Shake the SPME vials gently and softly (never spread the oil through the vial walls

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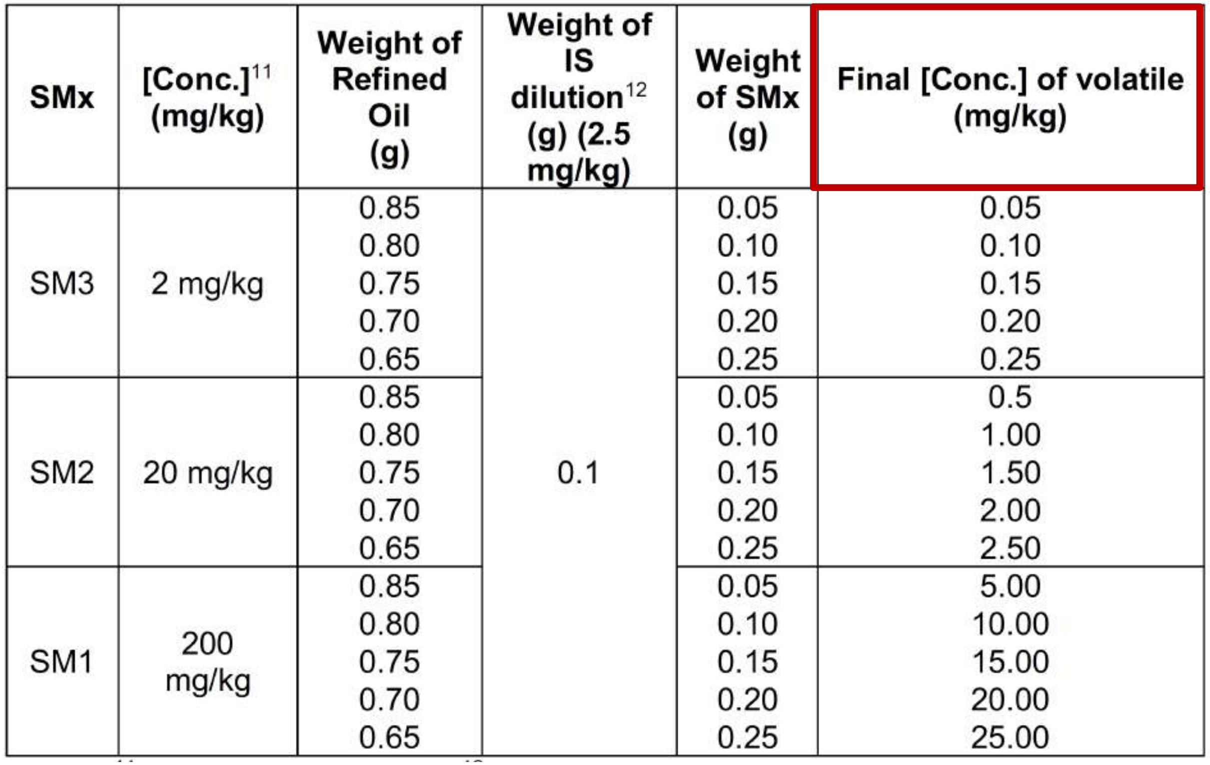
Preparation of the samples for building the calibration curves

Ready for GC analysis



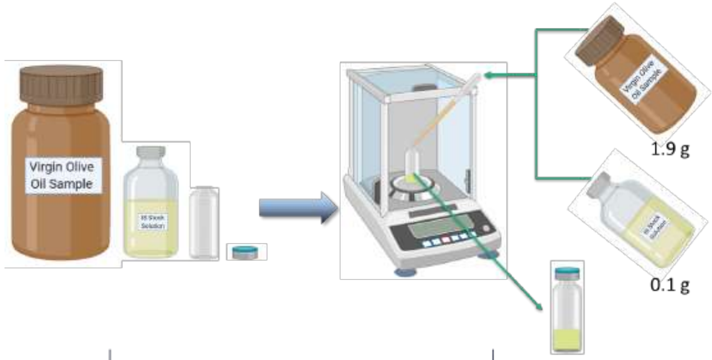
SMx	[Conc.][11] (mg/kg)	Weight of Refined Oil (g)	Weight of IS dilution[12] (g) (2.5 mg/kg)	Weight of SMx (g)	Final [Conc.] of volatile (mg/kg)
SM3	2 mg/kg	0.85	0.1	0.05	0.05
		0.80		0.10	0.10
		0.75		0.15	0.15
		0.70		0.20	0.20
		0.65		0.25	0.25
SM2	20 mg/kg	0.85		0.05	0.5
		0.80		0.10	1.00
		0.75		0.15	1.50
		0.70		0.20	2.00
		0.65		0.25	2.50
SM1	200 mg/kg	0.85		0.05	5.00
		0.80		0.10	10.00
		0.75		0.15	15.00
		0.70		0.20	20.00
		0.65		0.25	25.00

Example SMA

Sequence of the analysis

1. Blank (Empty vial)
2. Blank of the matrix
(Refined olive oil "2.0 g")
3. Blank of the matrix + IS
(Refined olive oil "2.0 g" + IS "0.1 g")
4. Blank (Empty vial)
5. 0.05 mg/kg vial
6. 0.1 mg/kg vial
7. 0.15 mg/kg vial
8. 0.20 mg/kg vial
9. Blank (Empty vial)
10. 0.25 mg/kg vial
11. 0.5 mg/kg vial
12. 1 mg/kg vial
13. 1.5 mg/kg vial
14. Blank (Empty vial)
15. 2 mg/kg vial
16. 2.5 mg/kg vial
17. 5 mg/kg vial
18. 10 mg/kg vial
19. Blank (Empty vial)

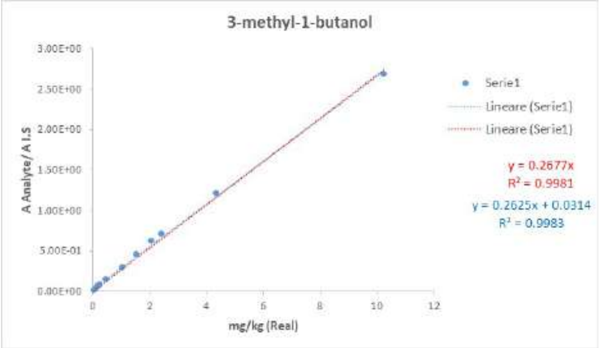
Sample preparation

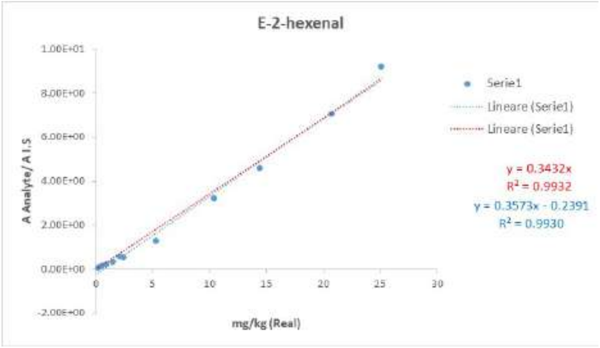


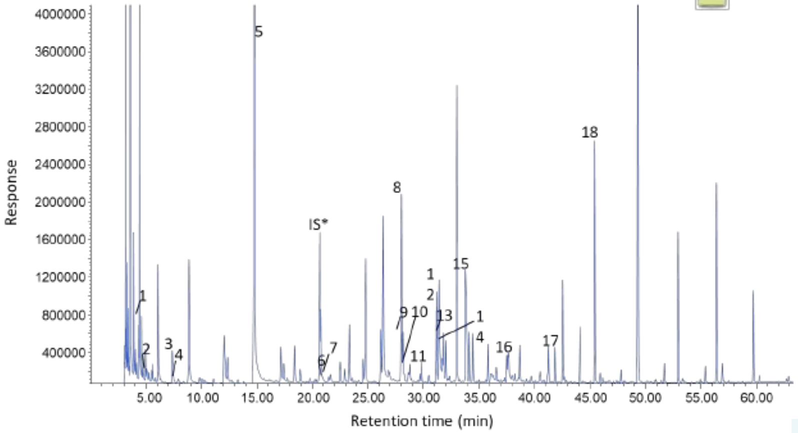
Virgin olive oil sample

I.S. 4-methyl-2-pentanol

Calibration solutions
(18 VOCs)







Chromatographic areas

Sample → Chromatographic areas → Quantification **(Calibration)** → Concentration (mg/kg)

I.S.

3 Quantification Methods (QM1, QM2, QM3)

- **Quantification method 1 (QM1):** Data obtained using the calibration curve $A_{Analyte}/A_{IS}$ vs. concentration (regression line in the form of **Y=mX**).

- **Quantification method 2 (QM2):** Data obtained using the calibration curve $A_{Analyte}$ vs. concentration (regression line in the form **Y=mX**).

- **Quantification method 3 (QM3):** Data obtained using the calibration curve of the IS and the analyte. This third method has been reported by Kalua et al., 2006. It corresponds to the following procedure:

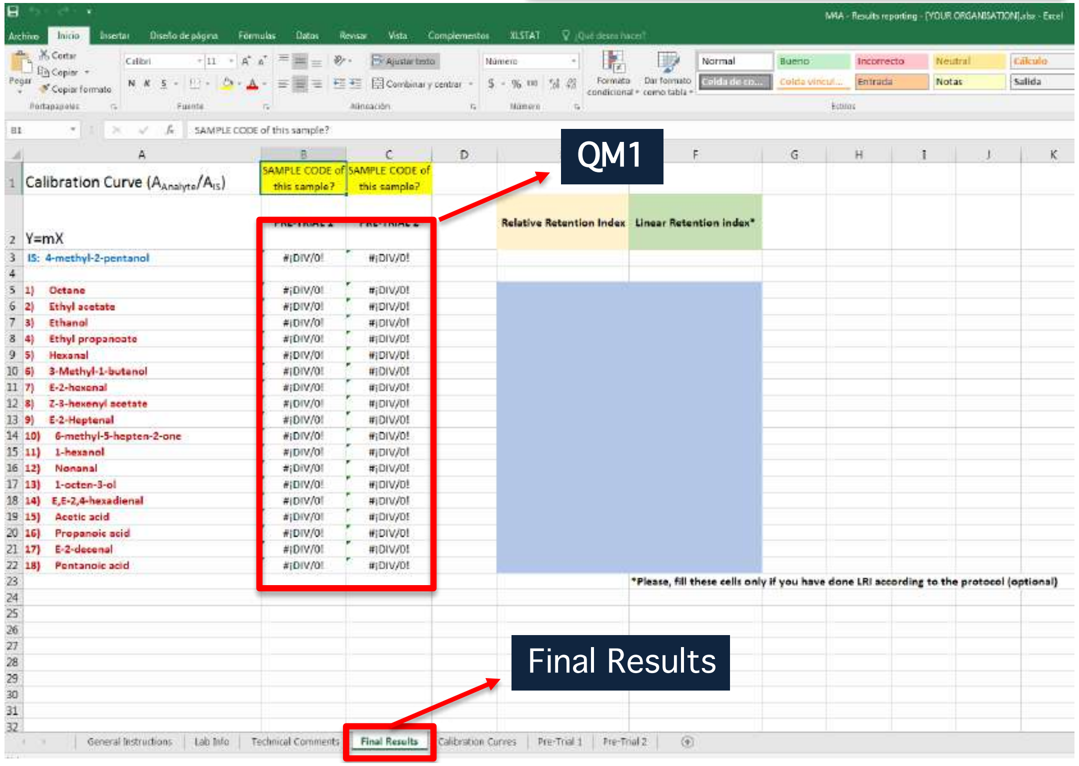
$$C_{Analyte} = ((A_{Analyte} * C_{IS}) / A_{IS})) * (m_{IS}/m_{Analyte})$$

18 compounds
X
2 detectors (FID/MS)
X
3 quantification methods
X
6 parameters

648 values

- Repeatability, *r within batch*
- Reproducibility, *R between batch*
- Linearity
- Recovery
- LOD
- LOQ

\+ Precision (IS)



	A	B	C	D	E	F
1	Calibration Curve ($A_{Analyte}/A_{IS}$)	SAMPLE CODE of this sample?	SAMPLE CODE of this sample?			
2	Y=mX	PRE-TRIAL 1	PRE-TRIAL 2		Relative Retention Index	Linear Retention index*
3	IS: 4-methyl-2-pentanol	#¡DIV/0!	#¡DIV/0!			
4						
5	1) Octane	#¡DIV/0!	#¡DIV/0!			
6	2) Ethyl acetate	#¡DIV/0!	#¡DIV/0!			
7	3) Ethanol	#¡DIV/0!	#¡DIV/0!			
8	4) Ethyl propanoate	#¡DIV/0!	#¡DIV/0!			
9	5) Hexanal	#¡DIV/0!	#¡DIV/0!			
10	6) 3-Methyl-1-butanol	#¡DIV/0!	#¡DIV/0!			
11	7) E-2-hexenal	#¡DIV/0!	#¡DIV/0!			
12	8) Z-3-hexenyl acetate	#¡DIV/0!	#¡DIV/0!			
13	9) E-2-Heptenal	#¡DIV/0!	#¡DIV/0!			
14	10) 6-methyl-5-hepten-2-one	#¡DIV/0!	#¡DIV/0!			
15	11) 1-hexanol	#¡DIV/0!	#¡DIV/0!			
16	12) Nonanal	#¡DIV/0!	#¡DIV/0!			
17	13) 1-octen-3-ol	#¡DIV/0!	#¡DIV/0!			
18	14) E,E-2,4-hexadienal	#¡DIV/0!	#¡DIV/0!			
19	15) Acetic acid	#¡DIV/0!	#¡DIV/0!			
20	16) Propanoic acid	#¡DIV/0!	#¡DIV/0!			
21	17) E-2-decenal	#¡DIV/0!	#¡DIV/0!			
22	18) Pentanoic acid	#¡DIV/0!	#¡DIV/0!			

*Please, fill these cells only if you have done LRI according to the protocol (optional)

General Instructions | Lab Info | Technical Comments | Final Results | Calibration Curves | Pre-Trial 1 | Pre-Trial 2

Published papers

SCAN ME

Food Control 123 (2021) 107823

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Food Control

journal homepage: www.elsevier.com/locate/foodcont

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Peer inter-laboratory validation study of a harmonized SPME-GC-FID method for the analysis of selected volatile compounds in virgin olive oils

Enrico Casadei[a], Enrico Valli[a], Ramón Aparicio-Ruiz[b,*], Clemente Ortiz-Romero[b], Diego L. García-González[b], Stefania Vichi[c], Beatriz Quintanilla-Casas[c], Alba Tres[c], Alessandra Bendini[a], Tullia Gallina Toschi[a]

- **Using FID:**
  - **CSIC** - Instituto de la Grasa, Sevilla, Spain.
  - **UNIBO** - Alma Mater Studiorum - Università di Bologna, Bologna, Italy.
  - **UB** - Universitat de Barcelona, Santa Coloma de Gramenet, Spain.

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Food Control 135 (2022) 108756

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journal homepage: www.elsevier.com/locate/foodcont

ELSEVIER

Collaborative peer validation of a harmonized SPME-GC-MS method for analysis of selected volatile compounds in virgin olive oils

Ramón Aparicio-Ruiz[a], Clemente Ortiz Romero[a], Enrico Casadei[b], Diego L. García-González[a], Maurizio Servili[c], Roberto Selvaggini[c], Florence Lacoste[d], Julien Escobessa[d], Stefania Vichi[e], Beatriz Quintanilla-Casas[e], Pierre-Alain Golay[f], Paolo Lucci[g], Erica Moret[g], Enrico Valli[b,*], Alessandra Bendini[b], Tullia Gallina Toschi[b]

- **Using MS:**
  - **UNIPG** - Department of Agricultural, Food and Environmental Sciences, Università degli Studi di Perugia, Perugia, Italy.
  - **ITERG** - Institut des Corps Gras, Canejan, France.
  - **UB** - Universitat de Barcelona, Santa Coloma de Gramenet, Spain.
  - **UNIUD** - Department of Agri-Food, Animal and Environmental Science Università degli Studi di Udine, Udine, Italy.
  - **Nestlé** - Research Center, Lausanne, Switzerland.

Summary results

18 compounds
X
2 detectors (FID/MS)
X
3 quantification Methods
X
6 quality parameters

648 values

7 OLEUM partners

Linearity (R^2)
FID 0.96 (0.94-0.99)
MS 0.98 (0.94-0.99)

Repeatability (RSD%)
FID 11.53 (6.50-15.60)
MS 7.60 (3.89-17.23)

Reproducibility (RSD%)
FID 39 (12-122) → Ethyl propanoate (below LOQ)
MS 31 (13-64)

Recovery (%)
FID 89 (50-160)
MS 94 (72-106)

Precision IS (RSD%)
FID 7.56
MS 9.66

LOD (mg/kg)
FID 0.08 (0.01-0.6)
MS 0.03 (0.01-0.18) → (*E*)-2-decenal

LOQ (mg/kg)
FID 0.246 (0.01-1.93)
MS 0.08 (0.01-0.53)

Validation Pipeline

Round table – Premises

OLEUM partners – In house validation

Format
ISO 78-2, 2016

OLEUM Peer interlab. validation

7 Laboratories – Pre Trial

2 Test materials

2nd OLEUM Workshop:
“Hands on New Analytical Method for Quality & Authenticity of Olive Oil” – Workshop

Feedback, critique of the method

20 Laboratories – Trial Proper

10 Test materials

Work in progress – Definition of limits and ranges

Oleum

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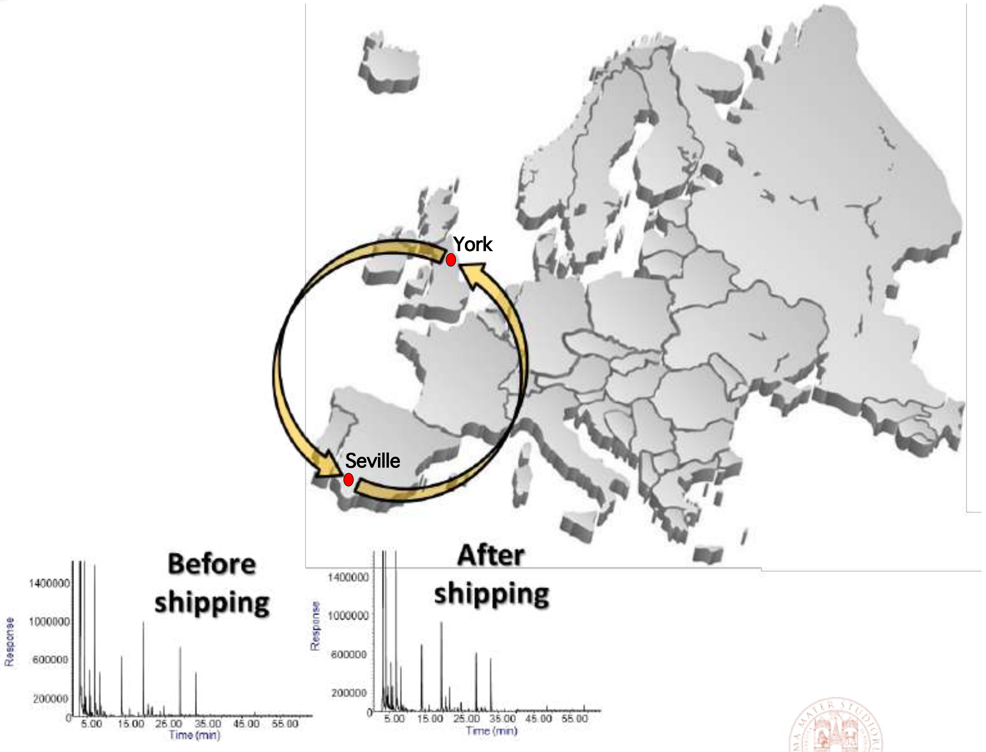
OLEUM Interlaboratory validation process

Following the **IUPAC** Protocol for the design, conduct and interpretation of method-performance studies*.

Trial proper → 18 VOCs x 2 Detectors (**FID, MS**)

Same sample for MS and FID !



- ✓ 20 labs (from Europe, UK, US, China and Japan) took part in the study and received 10 test materials comprising 5 sets of individually numbered blind duplicates.
- ✓ Participants were sent a practice sample where the approximate concentration of the sample was provided. Samples were prepared in bulk by CSIC and then sent to Fera Science Ltd for subsampling, labelling and dispatch to participants.
- ✓ The samples used for this validation study were selected to be above the mean concentration for each one of the 18 compounds. It was necessary to blend real EVOO/VOO/LOO in order to cover the natural concentrations of the 18 analytes within 5 paired samples, this resulted in some compounds being present at concentrations lower than the LOQ.

*Pure & Appl. Chem., Vol. 67, No. 2, pp. 331-343, 1995

☑ Some evidence from the validation process

		Mean RSD$_R$% pairs	**RSD$_R$% 1&8**	**RSD$_R$% 2&4**	**RSD$_R$% 3&11**	**RSD$_R$% 6&7**	**RSD$_R$% 10&12**
1)	Octane	27.74	21.90	30.50	23.60	28.10	34.60
2)	Ethyl acetate	15.90	12.40	8.50	12.10	23.90	22.60
3)	Ethanol	23.76	27.10	53.50	8.00	13.00	17.20

Summary results (RSD$_R$%) of the statistical elaboration relating to the FID method.

In the interlaboratory validation process, the RSD$_R$ values were lower for FID method than MS in 11 compounds.

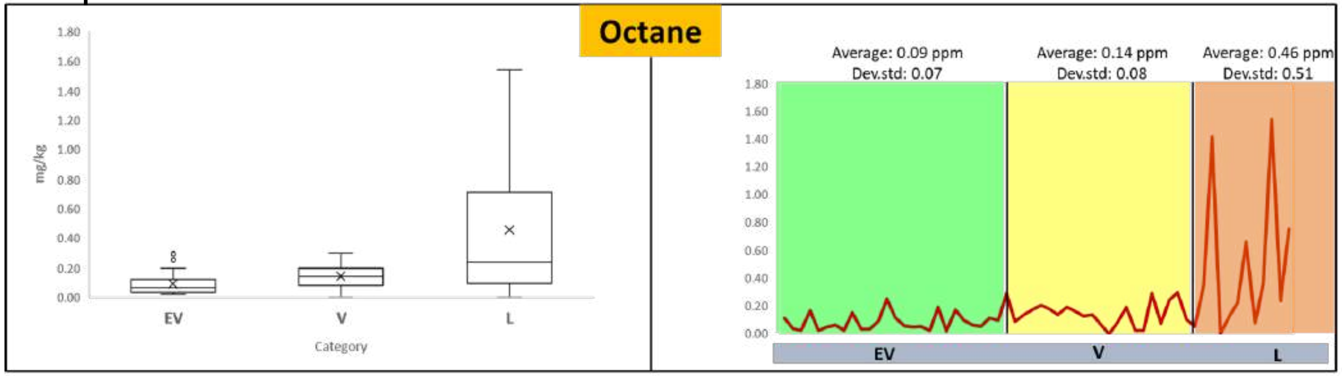
The mean concentrations obtained with FID and MS were similar. However, in general terms, the FID provided better results in terms of reproducibility than the MS method.

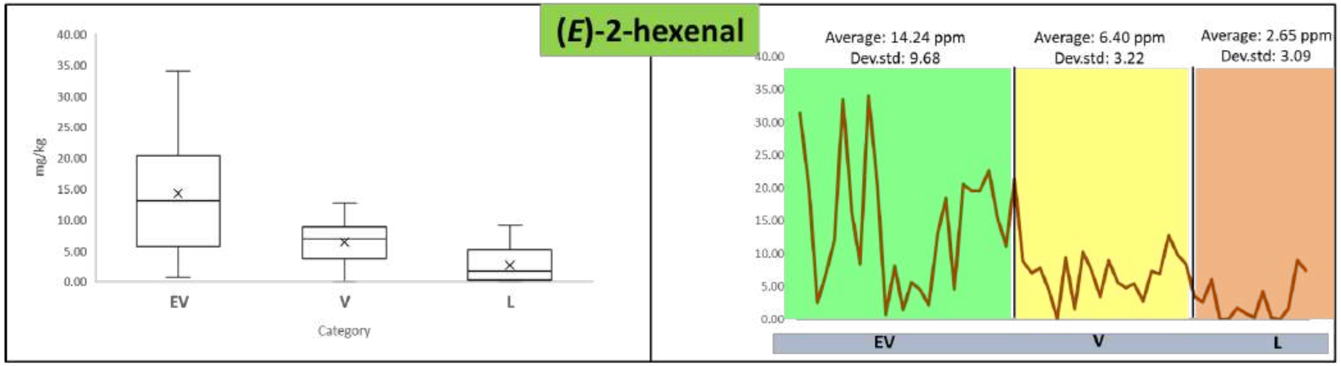
The observation of a different reproducibility for both detectors agrees with our previous experience when carrying out a peer interlaboratory study within OLEUM partners.

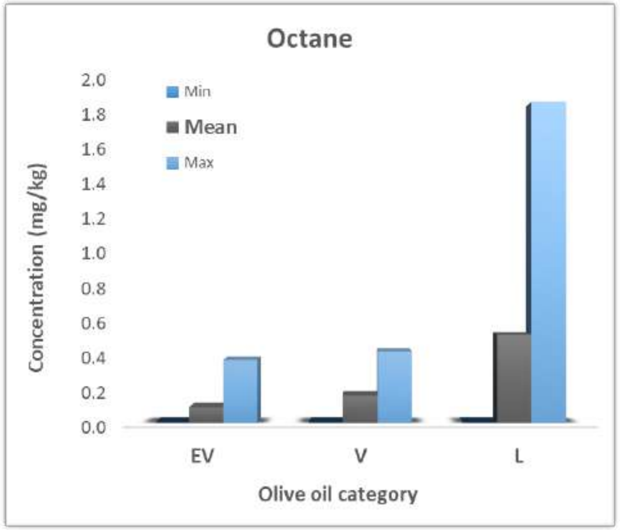
It is important to consider the advantages and disadvantages in the use of the two detectors.

Future developments

Collection of data in order to establish limits and ranges of volatile compounds.







Definition of limits and ranges

- Reliable quantification data
- Representative samples

  (covering categories, defects, and wide range of concentration values).
- Interlab. perspective

Concluding remarks

- ✓ We have an information base of all the error sources and the performance of the method with two possible detectors (FID and MS) and with an interlaboratory perspective.

- ✓ Other information base is being considered at the moment: FID/MS comparison, LOQ/Odour threshold relationship and the concentration ranges in virgin olive oils in a large sample set (categories, cultivars, defects, etc.).

- ✓ The application of the method can be addressed to:

  - ➢ Support in conflicts/litigations between sensory panels.

  - ➢ Support daily work of the panels (e.g. priorization, doubtful samples, borderline samples).

  - ➢ Calibration/support of other rapid/screening instrumental techniques.

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