Additional data on the application of the OLEUM method to quantify volatiles molecules

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Civil Dialogue Group (CDG) on Olive Oil and Olives, October 27th, 2023

THE FRAMEWORK

Multiple perspectives

Scientific community

- Extraction capacity
- Maximum information
- Sensitivity

Olive oil actors

- Simplicity
- Easy to apply
- Rapid
- Understandable

Validation (Fit for purpose)

Regulation bodies

- Clear definition of purpose
- High applicability
- Low error
- Highly distributed
- Internationally accepted

OLEUM Project: An harmonized and validated protocol for the quantification of volatile markers

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

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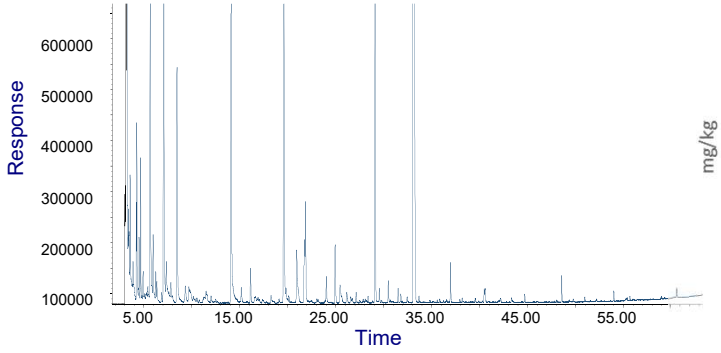
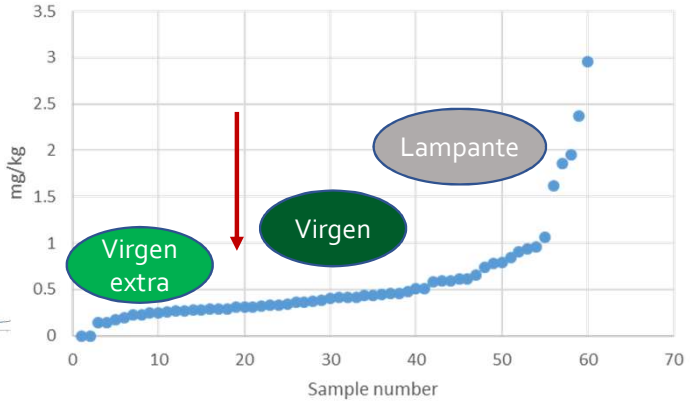
a. Protocol for SPME-GC-FID (VERSION A)

b. Protocolo for SPME-GC-MS (VERSION B)

c. Guide document for building calibración curves

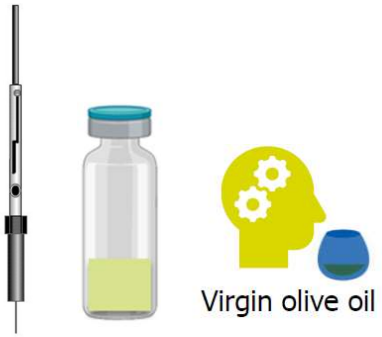




THE FRAMEWORK

The **targeted** and **quantitative instrumental analysis of volatiles** (SPME-GC-FID/MS), **fully validated during the OLEUM project**, must serve:

1) to be applied to **borderline EVOOs/VOOs** and thus:
2) to **provide a classification** in case of **disagreement between panels**



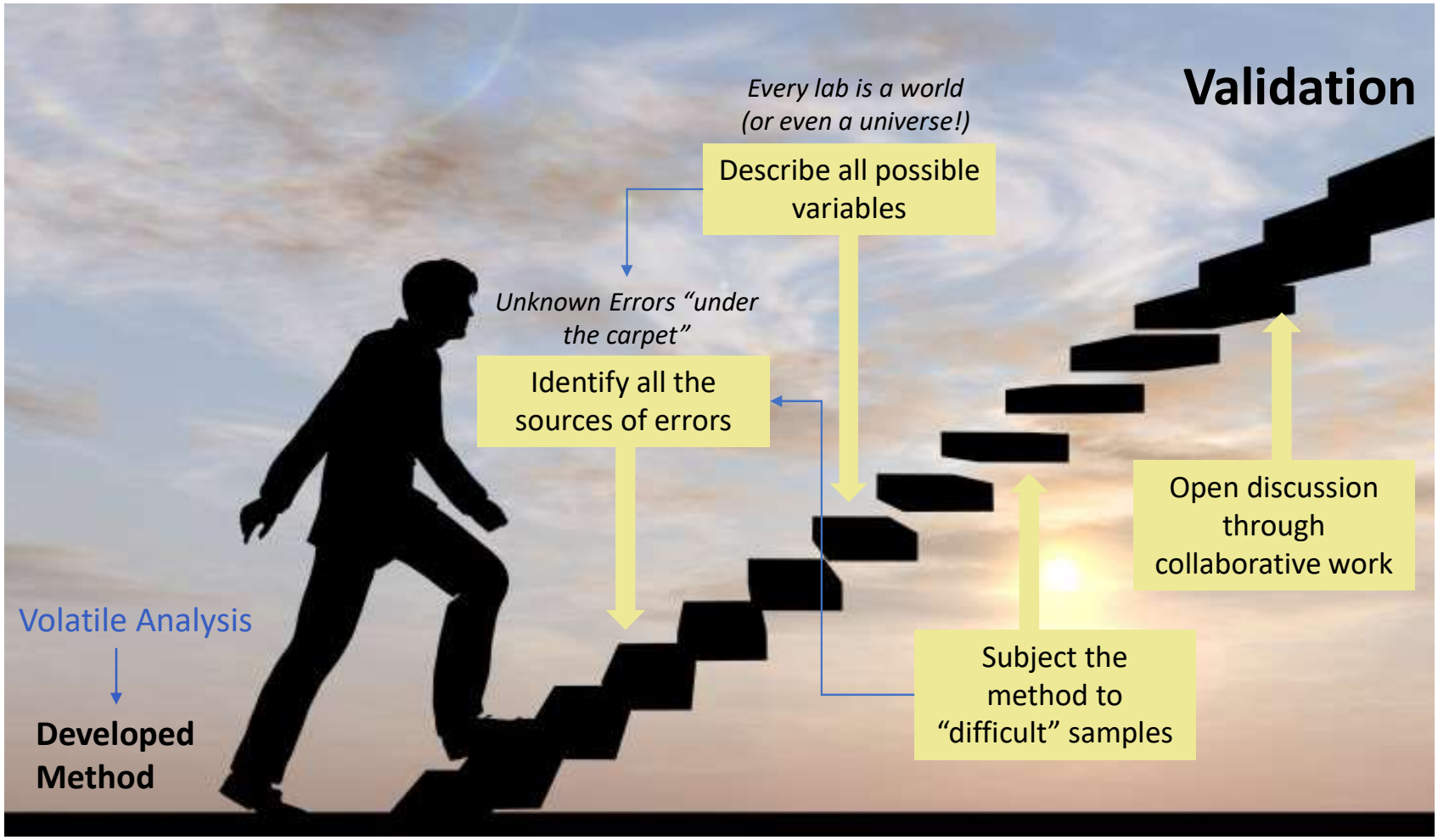
It is important to underline that:

In **no case is it in competition with the Panel test** but **it will serve as a support**.

The **interaction between chemical and organoleptic experts of the IOC** is extremely relevant to correctly introduce the method into the legislation. once it will be fully implemented and verified.

The **final goal** is to **reduce organoleptic non-conformities**. without weakening the Panel test and its meaning for the definition of the quality of EVOOs/VOOs. but. on the contrary. strengthening it.

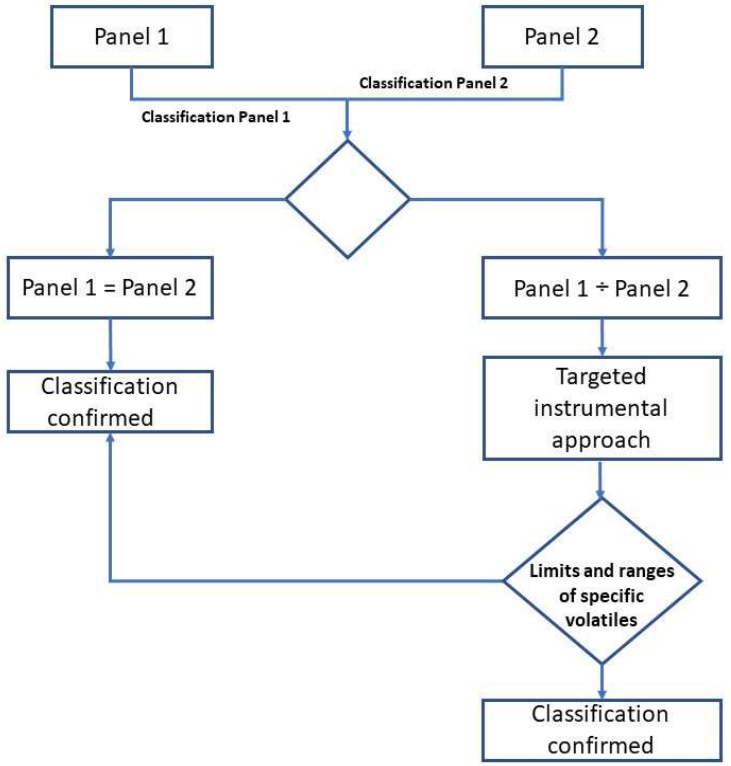
Analytical Determination of Volatile Compounds: Is the way to validation a rapid procedure?



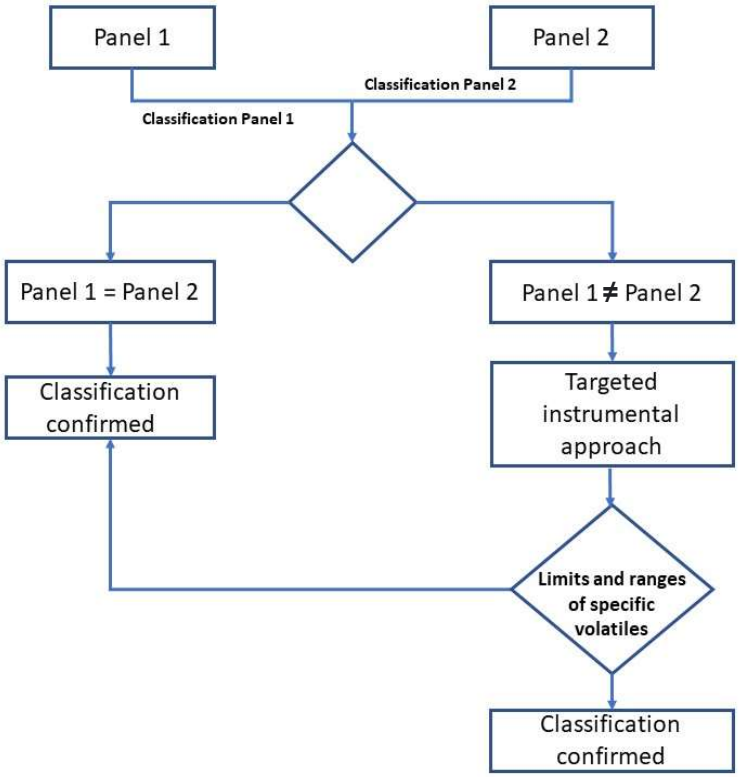
The framework: hypothesis of use of targeted approaches to determine volatiles for the classification (Panel disagreement)

COI/T20/Doc.15 Rev. 10-2018
§10.6

Should the panel not confirm the declared category as regards the organoleptic characteristics. the interested party may request the national authorities or their representatives to have carried out without any delay **two independent counter-assessments** by two other panels recognised by the IOC or approved by the competent authorities at national level. The characteristics concerned shall be deemed consistent with the characteristics declared if **both counter-assessments confirm the declared category**. If that is not the case. the interested party shall be responsible for the cost of the two counter-assessments.



More in general a **joint strategy** able **to combine sensory and instrumental data** useful **in cases of disagreement between** two **panels** is needed



Possible use of a targeted instrumental approach as a **confirmation/disconfirmation tool** of the sensory assessment (Panel Test)

Targeted methods (SPME-GC-FID/MS)

↓

Official control labs

Screening methods

↓

Quality assurance labs/Official control labs

The 18 volatile compounds quantified

(minimum number of highly diagnostic sensory markers)

Oleum

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

Oleum

1 procedure
2 detectors (FID and MS)

Measurand: 18 selected volatile compounds (VOCs) in virgin olive oils (in mg/kg).

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

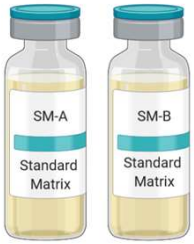
18 VOCs

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

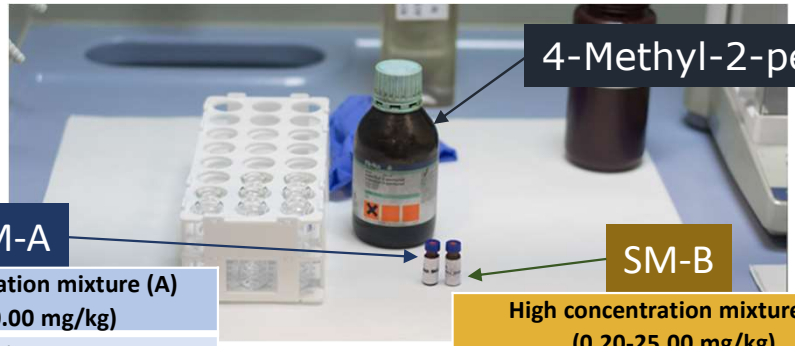
1. Octane
2. Ethyl acetate
3. Ethanol
4. Ethyl propanoate
5. Hexanal
6. 3-Methyl-1-butanol
7. (*E*)-2-Hexenal
8. (*Z*)-3-Hexenyl acetate
9. (*E*)-2-Heptenal
10. 6-Methyl-5-hepten-2-one
11. 1-Hexanol
12. Nonanal
13. 1-Octen-3-ol
14. (*E.E*)-2.4-Hexadienal
15. Acetic acid
16. Propanoic acid
17. (*E*)-2-Decenal
18. Pentanoic acid

*Internal standard: 4-methyl-2-pentanol

2 Standard mixtures to simplify the analysis: 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

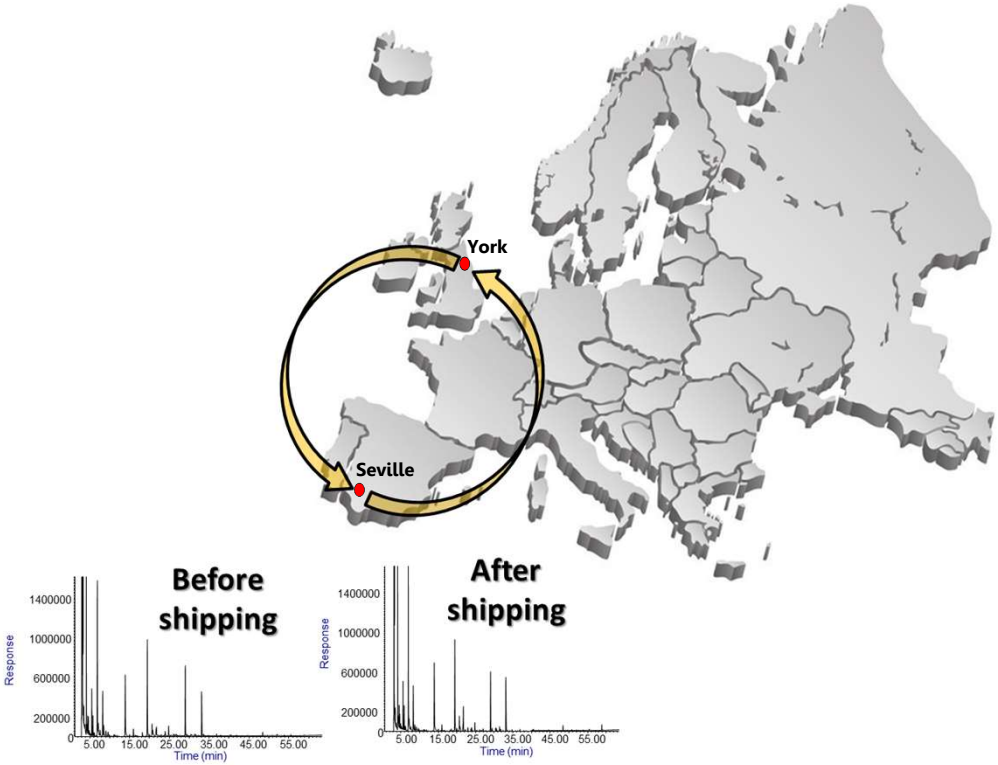
OLEUM Interlaboratory validation process

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

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

Same samples 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

Volatile compounds	Mean RSD_R%			
	OLEUM peer validation		OLEUM trial proper on 10 samples	
	FID	MS	FID	MS
Octane	12.00	38.50	27.74	39.12
Ethyl acetate	18.20	28.17	15.90	29.06
Ethanol	35.70	32.33	23.76	45.44

Summary results (RSD_R%) of the statistical elaboration relating to the OLEUM peer validation and trial proper.

In the interlaboratory validation process. the RSD_R values were lower for FID method than MS for 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 disseminate the advantages and disadvantages in the use of the two detectors.

THE IOC RING TEST

- **Objectives** of the **IOC ring test** were to 1) let the labs to have access to the method and make practice. 2) evaluate their proficiency (it is a new method in respect with those included in the standards 3) analyse the results in view of publishing the method and 4) after an extended application. fixing limits/ranges for some volatiles.

- **Two samples** were selected to determine the content of **18 volatiles compounds**. The analysis could be conducted using one or both of these **two detectors**: **FID and MS**.

- IOC decided to sent the call for the ring test within the **list of labs applying for the recognition program**.

- **GSC** was in charge of **samples preparation and distribution**.

Mean RSD_R% pairs IOC ring test on 2 samples in comparison with the OLEUM validation

Volatile compounds	Mean RSDR% pairs IOC ring test on 2 samples	
	FID	MS
Octane	10.55	28.78
Ethyl acetate	36.34	24.08
Ethanol	24.59	33.27
Ethyl propanoate	26.70	49.85
Hexanal	34.86	35.74
3-Methyl-1-butanol	48.57	26.09
(*E*)-2-hexenal	52.59	41.14
(*Z*)-3-hexenyl acetate	50.95	35.00
(*E*)-2-heptenal	42.55	26.23
6-methyl-5-hepten-2-one	35.17	33.62
1-hexanol	27.46	24.80
Nonanal	41.77	35.35
1-octen-3-ol	62.75	37.38
(*E.E*)-2.4-hexadienal	87.27	27.88
Acetic acid	33.69	24.26
Propanoic acid	17.69	29.03
(*E*)-2-decenal	72.07	40.21
Pentanoic acid	36.86	34.73
RSD < 40%	**10**	**15**

FID & MS compounds with RSD_R < 40%

Volatile compounds	FID	MS
Octane	10.55	28.78
Ethyl acetate	36.34	24.08
Ethanol	24.59	33.27
Hexanal	34.86	35.74
6-methyl-5-hepten-2-one	35.17	33.62
1-hexanol	27.46	24.80
Acetic acid	33.69	24.26
Propanoic acid	17.69	29.03
Pentanoic acid	36.86	34.73

- ✓ Focus on these **9 compounds** to analyze the results.
- ✓ No large errors associated to integration problems. difficulties in identification or other technical reason.

Validation results pairs IOC ring test on 2 samples

Volatile compounds	FID & MS compounds with RSDR < 40%					
	RSDR%		Mean Hor		Mean HoR	
	FID	MS	FID	MS	FID	MS
Octane	10.55	28.78	0.4	0.3	0.8	2.0
Ethyl acetate	36.34	24.08	0.5	0.4	3.5	1.7
Ethanol	24.59	33.27	0.6	0.4	3.4	3.3
Hexanal	34.86	35.74	0.5	0.3	2.9	2.6
6-methyl-5-hepten-2-one	35.17	33.62	0.7	0.4	2.3	1.9
1-hexanol	27.46	24.80	0.3	0.3	2.1	1.8
Acetic acid	33.69	24.26	0.5	0.4	2.7	2.0
Propanoic acid	17.69	29.03	0.7	0.4	1.4	2.1
Pentanoic acid	36.86	34.73	0.4	0.4	1.9	2.0

Observations from the IOC ring test

- **8 labs** submitted results **for FID** and **14 for MS**.
- **All 18 compounds** showed excellent repeatability results for both detectors (**RSD_r% < 10%**).
- **10 compouds** for **FID** and **15** for **MS** showed good reproducibility results (**RSD_R% < 40%**).
- **9 compounds** showed **RSD_R% < 40% for both detectors.**

Proposal for publication

- **Given the full validation of the methods** and the **results of the IOC ring test**. the method is consolidated in terms of procedure. SOP and Excel sheets.

- **Publication as a IOC method** (it would be the first method for determining volatile compounds published by a regulatory body).

Results of the ring test on the determination of volatile compounds by SPME-GC-FID/MS

(Tullia Gallina Toschi, Diego L. García González)

Presentation of the final report

How to publish

INTERNATIONAL
OLIVE
COUNCIL

COI/T.20/Doc. No 29/Rev.2
June 2022

ENGLISH
Original: ITALIAN

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DOCUMENT TO DECLARE THE USE OF IOC METHODS FOR PHENOLIC COMPOUNDS DETERMINATION

METHOD 1: COI/T.20/Doc. No 29/Rev.1 2017. DETERMINATION OF BIOPHENOLS IN OLIVE OILS BY HPLC

PURPOSE

This method describes a procedure for the extraction and HPLC quantification of biophenolic minor polar (BMP) compounds in olive oils, such as the natural and oxidised derivatives of oleuropein and ligustroside, lignans, flavonoids and phenolic acids. The range of measurement is from 30 mg/kg to 800 mg/kg.

To disseminate the procedure in the laboratories

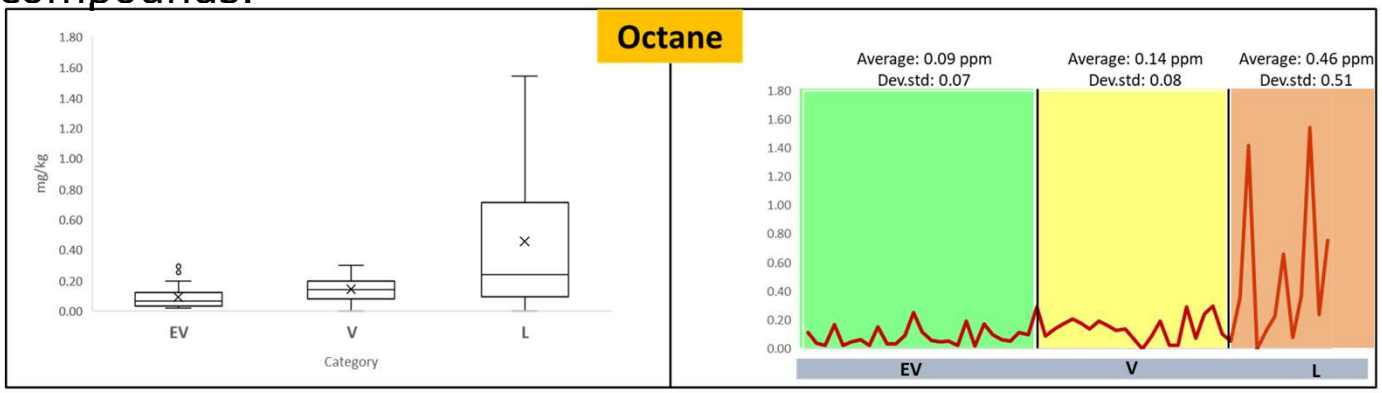
Method for the analysis of volatile compounds in virgin olive oil by SPME-GC-MS or SPME-GC-FID

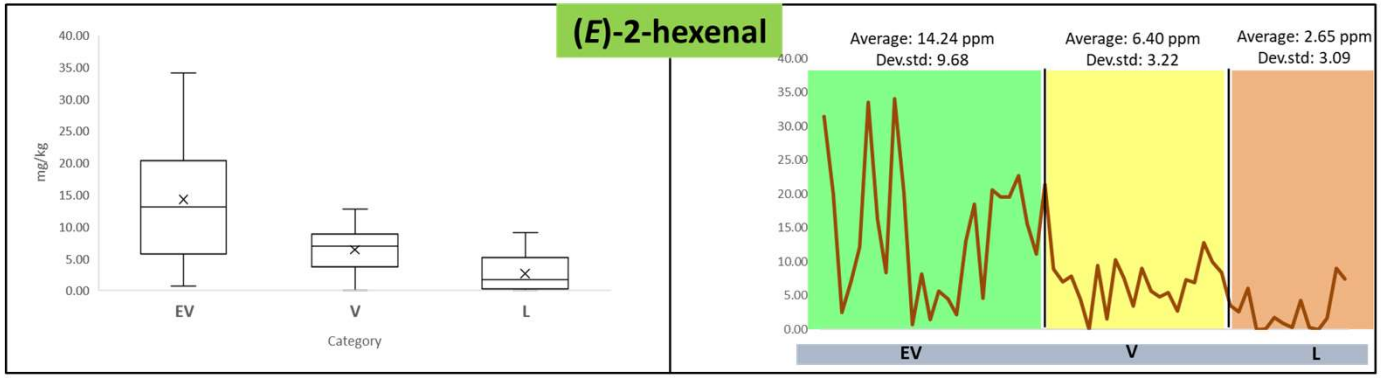
Draft of purpose of the method

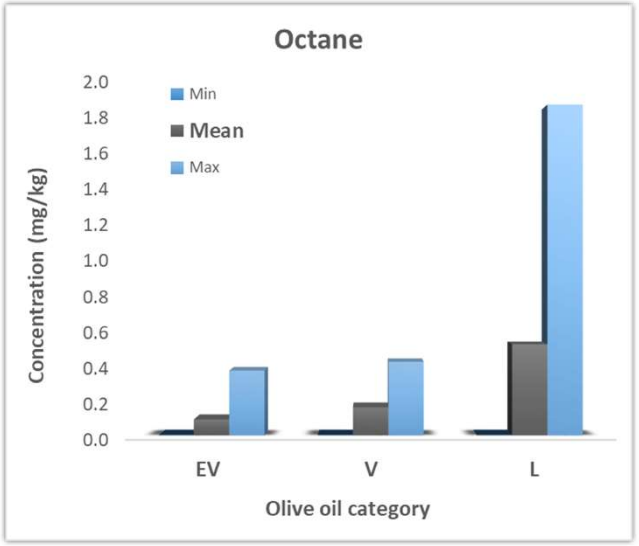
This method describes a procedure by SPME-GC-MS/FID for the quali-quantitative determination of selected volatile compounds (mg/kg) in virgin olive oils related to olfactory positive and negative sensory notes.

THE GOAL

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

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

OLEUM Full validation 20 Laboratories

Full validation

10 Test materials

Objective of IOC ring test on 2 samples:

1) Test the lab proficiency in applying the method(s)
2) Single markers proficiency (inter and intra-lab)

IOC ring test

2 Test materials

Publication as IOC method
Definition of limits and ranges

Examples of application of the method

❑ **SCIENTIFIC COLLABORATION AGREEMENT**

UNIBO and **Central Inspectorate for fraud repression** and quality protection of the Agrifood Products and Foodstuffs - Italian Ministry of Agriculture. Food Sovereignty and Forests (ICQRF).

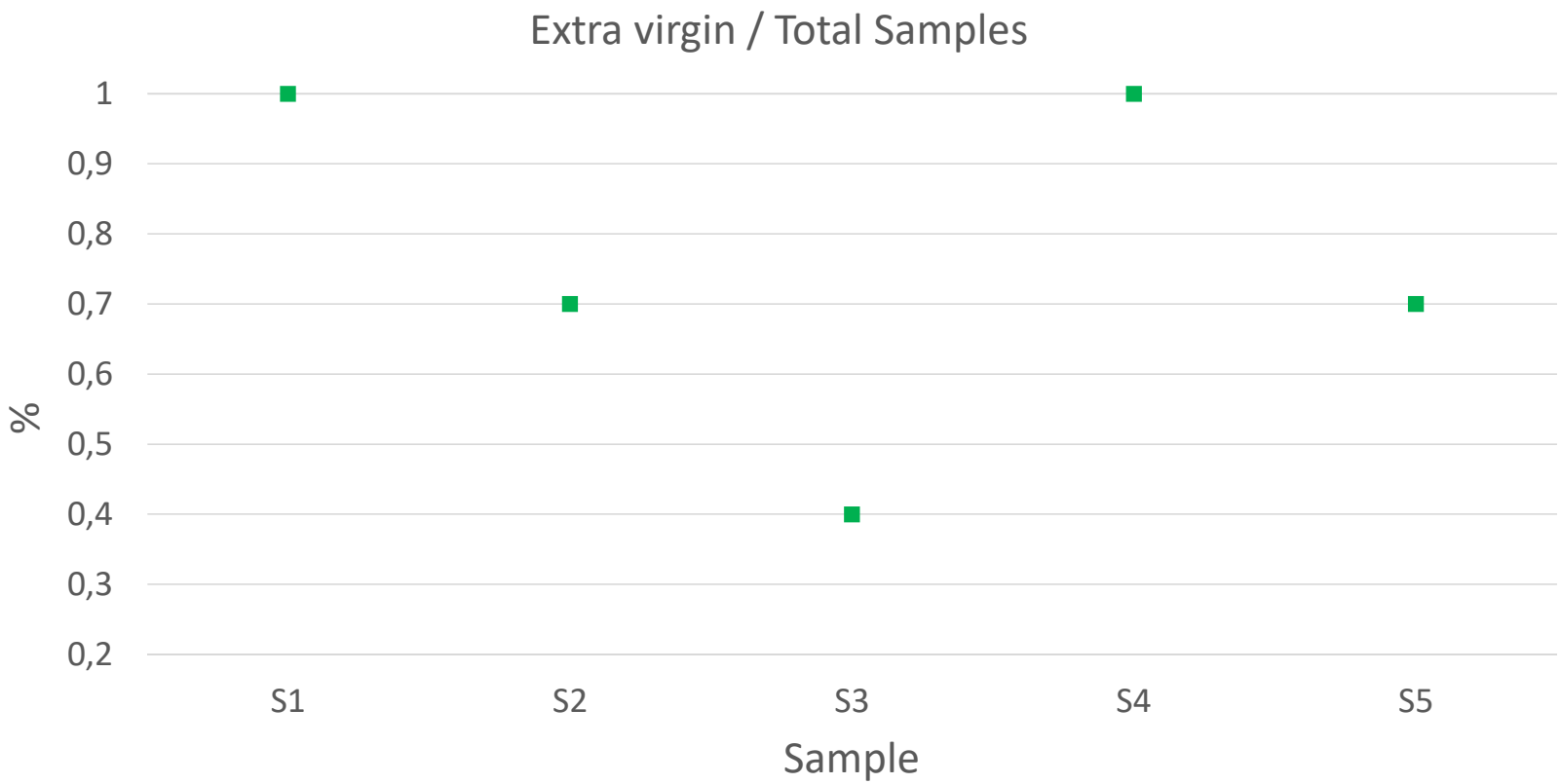
➢ Analysis of **53 virgin olive oils (14 EV, 24 V, 15 L)** by **SPME-GC-FID**

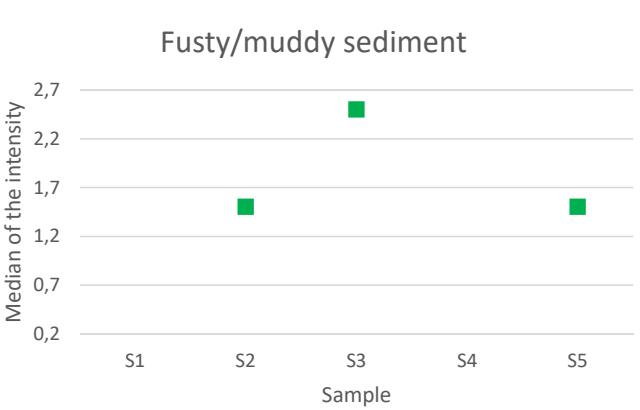
	Mean concentration (mg/kg)	
	***(E)*-2-hexenal**	***(Z)*-3-hexenyl acetate**
EV mean concentration	8.751	0.980
V mean concentration	0.898	0.653
L mean concentration	0.967	0.298

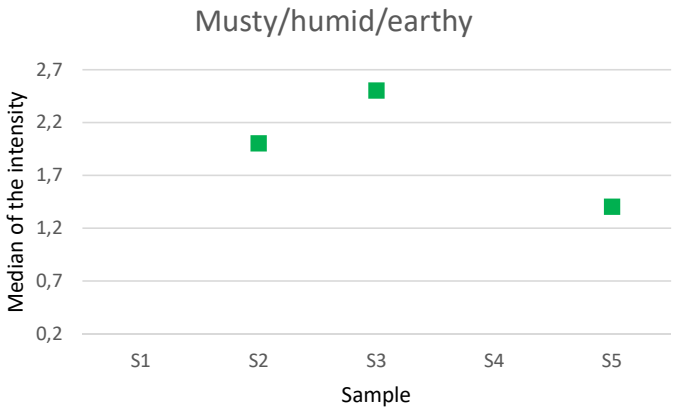
	Mean concentration (mg/kg)		
	Octane	**Ethanol**	**3-methyl-1-butanol**
EV mean concentration	0,111	2,717	0,328
V mean concentration	0,318	15,113	0,551
L mean concentration	3,223	21,668	2,109

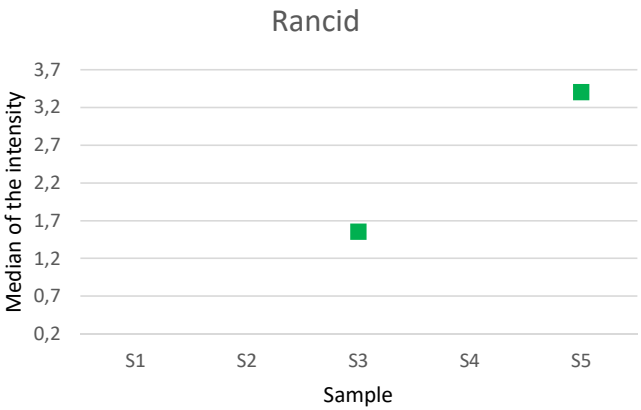
Examples of application of the method

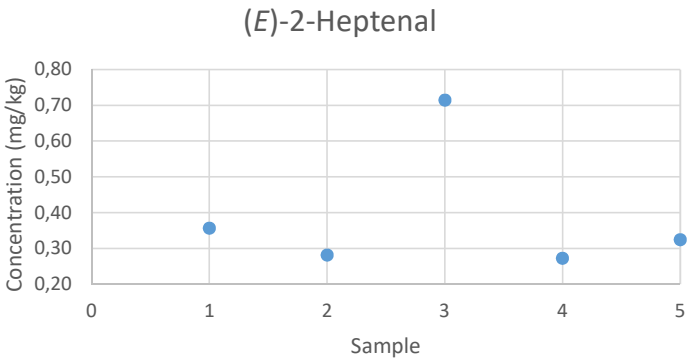
- **TUNISIAN SAMPLES (CHEMALI) SENSORY CLASSIFICATION**

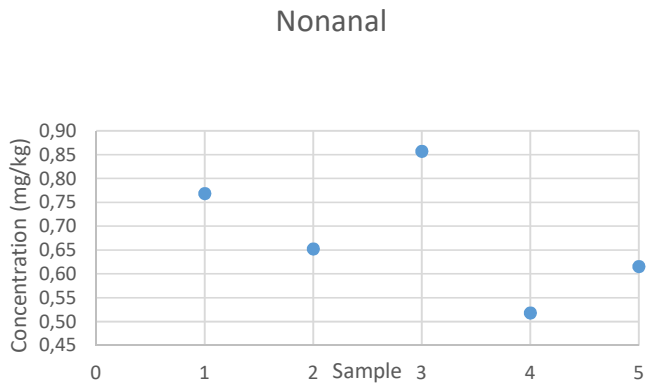


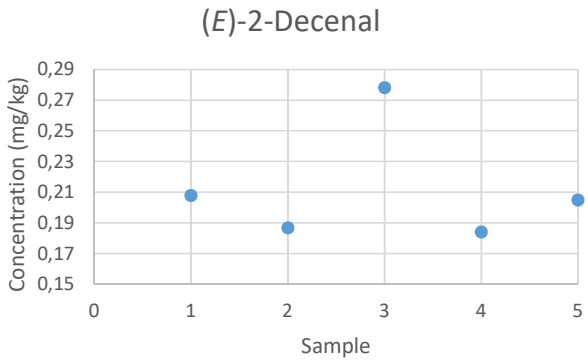

Fusty/muddy sediment
Median of the intensity
2,7
2,2
1,7
1,2
0,7
0,2
S1
S2
S3
S4
S5
Sample

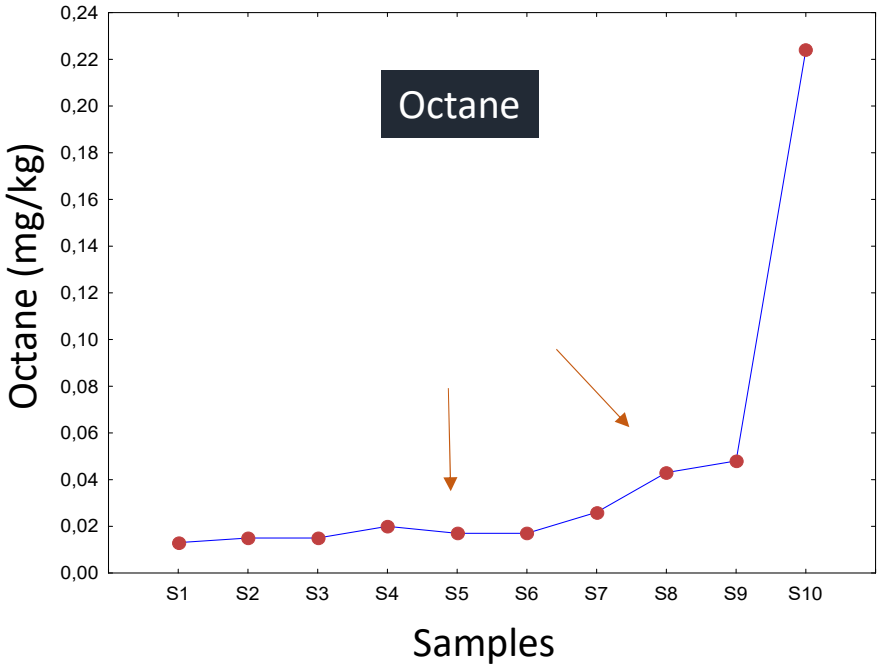

Musty/humid/earthy
Median of the intensity
2,7
2,2
1,7
1,2
0,7
0,2
S1
S2
S3
S4
S5
Sample

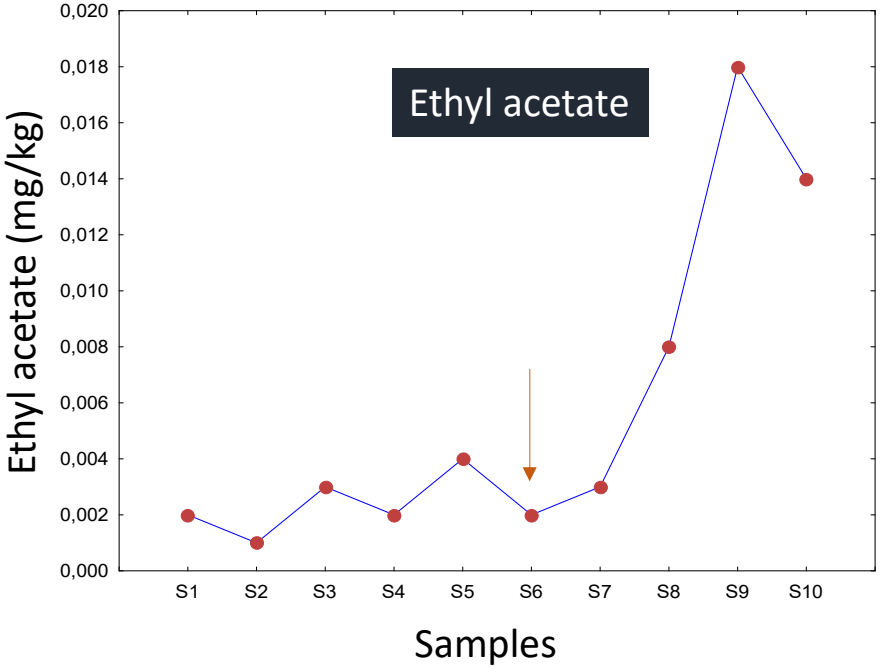

Rancid
Median of the intensity
3,7
3,2
2,7
2,2
1,7
1,2
0,7
0,2
S1
S2
S3
S4
S5
Sample


(E)-2-Heptenal
Concentration (mg/kg)
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0
1
2
3
4
5
Sample


Nonanal
Concentration (mg/kg)
0,90
0,85
0,80
0,75
0,70
0,65
0,60
0,55
0,50
0,45
0
1
2
Sample
3
4
5


(E)-2-Decenal
Concentration (mg/kg)
0,29
0,27
0,25
0,23
0,21
0,19
0,17
0,15
0
1
2
3
4
5
Sample


Octane
Octane (mg/kg)
0,24
0,22
0,20
0,18
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
Samples


Ethyl acetate
Ethyl acetate (mg/kg)
0,020
0,018
0,016
0,014
0,012
0,010
0,008
0,006
0,004
0,002
0,000
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
Samples

Conclusions

The method (targeted) is **fully validated** with two possible detectors (different costs and occurence) to be used by the **wider number of public and private quality control laboratories**

It is **ready to be published as official procedure** (IOC and EU)

It can be relatable with **screening and rapid procedures/tools** (not targeted) to evaluate the **risk** of a non correct classfication (preventive measures)

It can be applied to **borderline EVOOs/VOOs** and thus to **provide a classification** in case of **disagreement between panels**

It can reduce **organoleptic non-conformities** thus better:

1) Better **protect consumer** and virtuous and **honest producers**
2) Maintain **Europe's reputation as a leading light in the quality control of virgin olive oils** and a **forerunner** of innovative, diagnostic, easy to be applied and robust methods

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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]

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Method Article

Method for the analysis of volatile compounds in virgin olive oil by SPME-GC-MS or SPME-GC-FID☆,☆☆

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Multianalyte analysis of volatile compounds in virgin olive oils using SPME-GC with FID or MS detection: results of an international interlaboratory validation

Diego L. García-González, Enrico Casadei, Ramón Aparicio-Ruiz, Clemente Ortiz Romero, Enrico Valli, Paul Brereton, Anastasios Koidis, Martyna Korytkowska, Maurizio Servili ... See all authors

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