



## Analytical Methods

## An optimized method for sample preparation and elemental analysis of extra-virgin olive oil by inductively coupled plasma mass spectrometry

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## ARTICLE INFO

## Keywords:

ICP-MS  
 Sample preparation  
 Spectroanalytical technique  
 Toxic trace elements  
 Ultrasonic extraction  
 Virgin olive oil  
 Wet digestion

## ABSTRACT

The accurate determination of trace elements in vegetable edible oils is still an analytical challenge, owing to their low concentration levels and the complex matrix of the vegetable oils. The aim of this study was to develop a fast and simple analytical method to quantify 45 elements in small mass samples (0.5 g) of extra virgin olive oils by inductively coupled plasma mass spectrometry. To evaluate the best and fastest sample preparation procedure, ultrasonic extraction and wet digestion methods were compared using oil certified reference material with different reagent mixtures, reagent volumes, and times for sample extraction or digestion. The use of 5 mL reagent mixture F [10% (v/v) HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, 2:1 (v/v)] for sample digestion in a water bath (95 °C, 40 min) was found to produce satisfactory results in all cases as validated from sample recovery experiments over three different extra virgin olive oil samples.

## 1. Introduction

Olive oil is a major component of the Mediterranean diet. It is a rich source of antioxidants and monounsaturated fats, both of which are thought to protect cardiovascular health. However, olive oil can contain pollutants of various origins, including toxic elements. The presence of trace elements in vegetable edible oils may be due to soil composition and environmental pollution (Benincasa, Lewis, Perri, Sindona, & Tagarelli, 2007) as well as to contaminations during the oil production and conservation process (Matos Reyes & Campos, 2006). Elements that might be found at trace level in olive oil, such as Ca, Co, Cu, Fe, Mg, Mn and Ni, are known to have adverse effects on the flavor and oxidative stability of olive oil (Lepri et al., 2011; Matos Reyes & Campos, 2006), while other elements, such as As, Cr, Cd and Pb, are deleterious for their metabolic role and toxicity (Anthemidis, Arvanitidis, & Stratis, 2005; Huang & Jiang, 2001; Llorent-Martínez, Ortega-Barrales, Fernández-de Córdoba, & Ruiz-Medina, 2011). As quality criteria, the International Olive Council (IOC) has established a maximum residue level (MRL) for the content of As, Cu, Pb (0.1 mg kg<sup>-1</sup>) and Fe (3 mg kg<sup>-1</sup>) in olive oils and olive-pomace oils (International Olive Council, 2019). The maximum levels of Cu, and Fe in other vegetable oils have been also legislated (Codex Stan 33–1981), varying from 0.1 up to 5 mg kg<sup>-1</sup>.

Since the chemical quality of olive oils is related to the concentration of trace metals, the development of analytical procedures for the monitoring and control of their concentrations is very important and of great practical interest (Cabrera-Vique, Bouzas, & Oliveras-López, 2012).

Inductively coupled plasma optical emission spectrometry (ICP-OES) (Bakircioglu, Kurtulus, & Yurtsever, 2013), inductively coupled plasma mass spectrometry (ICP-MS) (Beltrán, Sánchez-Astudillo, Aparicio, & García-González, 2015; Damak, Asano, Baba, Ksibi, & Tamura, 2019; Luka & Akun, 2019; Pošćić et al., 2019) and atomic absorption spectrometry (AAS) (Cassella, Brum, Lima, Caldas, & de Paula, 2011), especially in the electrothermal atomization mode (ETAAS) (Cabrera-Vique et al., 2012; López-García, Vicente-Martínez, & Hernández-Córdoba, 2014), can be considered as suitable analytical techniques for olive oils analysis, due to their wide availability and good sensitivity. Alternative analytical techniques that can be used for the same purpose are electrothermal vaporization ICP-MS (Huang & Jiang, 2001) and direct analysis combining laser ablation with ICP-TOF-MS (Bings, 2002).

The results obtained with AAS, ICP-OES, and ICP-MS may be affected by matrix and/or spectral interferences when the sample solutions contain a high amount of residual carbon compounds (RCC) (Grindlay, Mora, Loos-Vollebregt, & Vanhaecke, 2013). Sample preparation is a crucial and critical step for the analytical procedure. In particular, the

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Received 3 September 2020; Received in revised form 29 April 2021; Accepted 4 May 2021

Available online 9 May 2021

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elemental content in olive oils is difficult to analyze, since their matrix is complex and characterized by high viscosity and organic content. Moreover, some elements are present in olive oils at very low concentration levels (Cabrera-Vique et al., 2012; Llorent-Martínez et al., 2011; Llorent-Martínez, Ortega-Barrales, Fernández-de Córdoba, Domínguez-Vidal, & Ruiz-Medina, 2011). Therefore, different sample preparation and extraction techniques have been developed in order to remove the organic content of the edible oil samples before their analysis, including liquid–liquid extraction (Dugo, La Pera, Giuffrida, Salvo, & Lo Turco, 2004), dry ashing (Brkljača, Giljanović, & Prkić, 2013), microwave dry ashing (Ni, Tang, Liu, Shen, & Mo, 2015), microwave-assisted digestion (MAD) (Llorent-Martínez et al., 2011a, 2011b; Luka & Akun, 2019), microwave-induced combustion (Pereira et al., 2010), simple dilution with a suitable solvent (Cabrera-Vique et al., 2012; Chaves, de Loos-Vollebregt, Curtius, & Vanhaecke, 2011), acid extraction in vortexed samples (Ni, Tang, Yu, & Wang, 2016), ultrasonic acid extraction (USE) (Camin et al., 2010; Hill, 2015; Pošćić et al., 2019; Trindade, Dantas, Lima, Ferreira, & Teixeira, 2015) and emulsification (He, Chen, Zhou, Wang, & Liu, 2014; Kara, Fisher, & Hill, 2015). Among these, MAD is the most commonly used extraction technique. However, MAD methods are time-consuming and require large amounts of reagent (and consequently often use a high final dilution to reduce the acid content) (Damak et al., 2019; Lepri et al., 2011; Trindade et al., 2015). In contrast to this, ultrasonic acid extraction offers a simple, quick, and sensitive approach for the extraction of elements from edible oils (Pošćić et al., 2019).

The aim of this work was to evaluate a new fast and simple approach for sample preparation and elemental analysis of extra virgin olive oils (EVOO) by ICP-MS. The method described here was noticed to reduce acid volume, final dilution and the sample transfer steps, to prevent sample loss and to minimize volume transfer errors. A certified reference material (CRM) of oil and recovery experiments were used for method validation.

## 2. Material and methods

### 2.1. Instrumentation

A Bruker (Bremen, Germany) 820-MS quadrupole ICP-MS spectrometer equipped with a collision-reaction interface (CRI) and a glass nebulizer was used for the elemental analysis. CRI was used with He and H<sub>2</sub> (99.9995% purity; SOL Spa, Monza, Italy) as cell gases. In particular, 30 mL min<sup>-1</sup> He and 70 mL min<sup>-1</sup> H<sub>2</sub> were introduced to the sampler and skimmer cones, respectively. As, Cr, Fe, Mn, Se, and V were determined by CRI, while other elements were quantified in standard mode. Equipment optimization in terms of sensitivity, resolution and mass calibration was performed before each working session by monitoring signals produced by a multi-elemental solution containing Ba, Be, Ce, Co, In, Pb, Mg, Tl, and Th (at 0.005 mg L<sup>-1</sup> from 10.00 ± 0.05 mg L<sup>-1</sup>; Spectro Pure, Ricca Chemical Company, Arlington, TX, USA) in the graphics mode of analysis. The <sup>140</sup>Ce<sup>16</sup>O<sup>+</sup>/<sup>140</sup>Ce<sup>+</sup> ratio was used to check the level of oxide ions in the plasma, which might interfere in the determination of some elements. Moreover, instrumental parameters such as RF power and carrier gas flow were optimized and the level of doubly charged ion was monitored using the signal <sup>137</sup>Ba<sup>2+</sup>/<sup>137</sup>Ba<sup>+</sup>.

A Varian (Victoria, Australia) Vista-MPX CCD Simultaneous ICP-OES with axial view mode was used to determine RCC. Both ICP-MS and ICP-OES analyses were performed following the operating parameters shown in Tables S1 and S2, respectively.

An Arioso (Human Corporation, Seoul, Korea) Power I RO-UP Scholar UV deionizer system was used for the production of analytical reagent-grade water with a resistivity of 18.3 MΩ cm.

A temperature-controlled water bath (±0.2 °C), model WB12, was obtained by Argo Lab (Modena, Italy) and used for the sample digestion.

An Ulsonix (Germany) proclean 10.0 ultrasonic cleaner (10 l, ultrasonic power 240 W) was used for the sample extraction.

A Gibertini Elettronica (Milan, Italy) Europe 60 analytical balance (sensitivity, 0.1 mg) was used to weigh all samples.

### 2.2. Interferences

Quantification of some elements (such as As, Cr, Fe, Se, and V) with quadrupole ICP-MS can be affected by polyatomic interferences (May & Wiedmeyer, 1998). This can be solved through the use of CRI with He and H<sub>2</sub> as collision and reaction cell gases, respectively, or using the standard mode analysis with the application of corrective element equations. In this study, different isotopes and both element equations and CRI were used to correct the signal intensity for the presence of spectral interferences. The correction equations are shown below:

$$\begin{aligned} 51\text{V} &= 51\text{V} - 3.1081 * ?53 + 0.3524 * \text{Cr}52; \\ 75\text{As} &= 75\text{As} - 3.127 * (?77-0.815 * ?82); \\ 78\text{Se} &= 78\text{Se} - 0.03043 * \text{Kr}83 - 0.1869 * ?76; \\ 98\text{Mo} &= 98\text{Mo} - 0.1111 * \text{Ru}101 \end{aligned}$$

where is used the symbol “?” in place of an element symbol to specify an arbitrary *m/z*.

The content of oxides was daily kept under control below 1% by calculating the oxides percentage on CeO<sup>+</sup>/Ce<sup>+</sup> masses. To this end, a standard solution containing 0.005 mg L<sup>-1</sup> of Ba, Be, Ce, Co, In, Pb, Mg, Tl, and Th, in 1% HNO<sub>3</sub> was daily measured, and the optimization parameters were adjusted in order to select the best instrument performance (Table S1), according to the information provided by Bruker (oxide interference CeO<sup>+</sup>/Ce<sup>+</sup> <1%; doubly charged interference: Ba<sup>++</sup>/Ba<sup>+</sup> <3%, and sensitivity: 9Be > 25000 cps, 115In > 250000 cps, 232Th > 100000 cps).

The sample matrix and the C content can enhance or suppress the ICP signal and influence the reported concentration of some elements (Astolfi et al., 2018; Astolfi, Marconi, Protano, & Canepari, 2020). The RCC in final digests was < 60 mg kg<sup>-1</sup> and did not significantly interfere with the analysis according to previously reported results (Astolfi et al., 2018).

### 2.3. Reagents

All reagents used were of the highest available purity or at least analytical reagent grade.

Calibration standards and spiked samples were prepared from the 1000 mg L<sup>-1</sup> single-element standard solutions containing Ag, Ca, Ce, K, Na, P, Si, Sr, and Zn obtained by SCP Science (Baie-d'Urfé, Quebec, Canada), from the 1000 mg L<sup>-1</sup> single-element standard solutions containing B, Co, Mg, P, Tl, Dy, Nd, Pr, and Tb supplied by Merck KGaA (Darmstadt, Germany) and from the 1 mg L<sup>-1</sup> multi-element standard solution containing 27 elements (1.000 ± 0.005 mg L<sup>-1</sup> As, Al, Ba, Be, Bi, Cd, Cr, Cs, Cu, Ga, La, Li, Mn, Mo, Nb, Ni, Pb, Rb, Sb, Se, Sn, Te, Ti, Tl, U, V, W) purchased from Ultra Scientific/Agilent Technologies (North Kingstown, RI, USA).

A standard solution of Y (at 0.005 and 0.2 mg L<sup>-1</sup> from 1000 ± 2 mg L<sup>-1</sup>) purchased from Panreac Química (Barcelona, Spain), for both ICP-MS and ICP-OES, and single standard solutions of Sc, Rh, In, and Th (at 0.010 mg L<sup>-1</sup> from 1000 ± 5 mg L<sup>-1</sup>) from Merck KGaA (Darmstadt, Germany), for ICP-MS only, in 1% (v/v) HNO<sub>3</sub>, were employed as internal standards.

HNO<sub>3</sub> (67%) was purchased from Carlo Erba Reagents S.r.l. (Milan, Italy), HCl (36%) and H<sub>2</sub>O<sub>2</sub> (30%) from Promochem, LGC Standards GmbH (Wesel, Germany), and citric acid anhydrous (99.5%) from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Ar of 99.9995% purity was supplied by SOL Spa (Monza, Italy).

The oil used as CRM was Conostan S-21 (Lot number: 21550100) and was purchased from SCP Science (Baie D'Urfé, Canada).

Disposable graduated tubes (2.5–5–10 mL in polypropylene) were obtained from Artiglass S.r.l. (Due Carrare, PD, Italy).

Table 1

Tested sample treatment methods for elemental analysis in extra virgin olive oil samples by ICP-MS.

Methods	Ultrasonic extraction (USE) at 19 °C, Wet digestion (WD) at 95 °C
Sample mass (mg)	500
Time (min)	10, 20 or 40
Reagent mixtures	A = aqua regia B = HNO <sub>3</sub> C = HNO <sub>3</sub> and H <sub>2</sub> O <sub>2</sub> , 2:1 v/v D = 5% (v/v) HNO <sub>3</sub> E = H <sub>2</sub> O <sub>2</sub> F = 10% (v/v) HNO <sub>3</sub> and H <sub>2</sub> O <sub>2</sub> , 2:1 v/v
Reagent mixture volumes (mL)	0.5, 1 or 5
Sample dilution (CRM)	10 or 200 times with deionized water
Sample dilution (samples, mixture F, WD, 40 min)	no further dilution

#### 2.4. Sample preparation methods

Two types of sample preparation procedures were applied to determine elemental contents in oil CRM samples: wet digestion (WD) and ultrasonic extraction (USE) (Table 1). Both sample treatments were tested with oil CRM masses of around 0.5 g, directly weighed into 10 mL polypropylene tubes subsequently filled with six different reagent mixtures [A = aqua regia; B = HNO<sub>3</sub>; C = HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, 2:1 v/v; D = 5% (v/v) HNO<sub>3</sub>; E = H<sub>2</sub>O<sub>2</sub> and F = 10% (v/v) HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, 2:1 v/v] at different volumes (0.5, 1 and 5 mL). The mixture was thoroughly shaken and then immediately placed in a water bath at 95 °C or in an ultrasonic bath at room temperature (19 °C) to extract the elements from the oil CRM to the reagent solution. Both WD and USE methods were performed for 10, 20 and 40 min. Three replicate measurements were carried out for all sample preparation procedures. The solutions obtained from oil CRM sample preparation procedures were diluted to 10 mL with

Table 2

Isotope, internal standard, IDL ( $\mu\text{g kg}^{-1}$ ), MDL ( $\mu\text{g kg}^{-1}$ ), linearity range ( $\mu\text{g kg}^{-1}$ ), and summary of recovery percentage (R%) and precision [as coefficient of variation (CV%)] for each element in extra virgin olive oils (three different type) by WD with 5 mL reagent mixture F.

Isotope	Internal standard	IDL <sup>a</sup>	MDL <sup>b</sup>	Linearity range <sup>c</sup>		Spike - level 1 <sup>d</sup>			Spike - level 2 <sup>d</sup>		
				N	LQL-UQL	R% n = 3	CvR% Intra-day n = 3	CvR% Inter-day n = 9	R% n = 3	CvR% Intra-day n = 3	CvR% Inter-day n = 9
<sup>107</sup> Ag	<sup>115</sup> In	0.04	0.06	6	10–500	94–96	0.1–2.8	1.5–2.5	90–93	1.2–2.4	0.5–2.3
<sup>27</sup> Al	<sup>45</sup> Sc	6	9	4	50–500	108–110	3.8–7	4.3–22	98–106	3.6–5.2	4.9–11
<sup>75</sup> As	<sup>79</sup> Y	0.2	0.3	8	2.5–500	96–97	0.6–1.6	6.4–9.1	87–91	1.0–2.0	1.7–2.3
<sup>11</sup> B	<sup>45</sup> Sc	8	20	6	550–27500	105–110	1.4–3.4	2.6–3.9	95–100	1.4–1.7	2.1–5.2
<sup>137</sup> Ba	<sup>115</sup> In	0.2	0.7	3	100–500—107–113	0.5–2.8	2.7–12				
<sup>9</sup> Be	<sup>45</sup> Sc	0.003	0.004	8	2.5–500	98–100	1.8–2.1	1.7–3.1	94–98	0.5–3.5	0.7–4.0
<sup>209</sup> Bi	<sup>232</sup> Th	0.01	0.1	8	2.5–500	100–102	0.2–3.2	1.1–2.9	96–102	1.3–2.4	1.8–5.5
<sup>44</sup> Ca	<sup>79</sup> Y	130	510	3	5000–20000—85–94	2.0–6.1	2.9–17				
<sup>112</sup> Cd	<sup>115</sup> In	0.02	0.07	8	2.5–500	92–96	0.4–0.6	1.7–2.9	91–93	0.1–1.6	1.0–2.5
<sup>140</sup> Ce	<sup>115</sup> In	0.03	0.1	8	2.5–500	98–101	0.4–1.4	1.0–3.4	96–96	0.4–1.8	1.4–2.5
<sup>59</sup> Co	<sup>45</sup> Sc	0.01	0.05	8	2.5–500	98–99	1.5–2.4	1.7–5.5	99–102	0.4–3.2	4.3–6.5
<sup>52</sup> Cr	<sup>79</sup> Y	0.2	0.3	8	2.5–500	101–103	1.0–1.9	1.6–6.9	97–101	0.7–2.7	1.9–4.5
<sup>133</sup> Cs	<sup>115</sup> In	0.004	0.007	8	2.5–500	96–102	0.5–1.1	1.2–2.9	94–97	0.2–2.6	2.3–3.9
<sup>65</sup> Cu	<sup>79</sup> Y	0.1	0.6	8	2.5–500	103–108	1.1–1.5	1.4–4.3	97–98	0.0–4.3	1.4–5.1
<sup>163</sup> Dy	<sup>115</sup> In	0.002	0.005	8	2.5–500	93–99	0.7–2.1	1.8–3.4	92–95	0.2–2.4	1.5–3.1
<sup>57</sup> Fe	<sup>79</sup> Y	7	12	5	100–2000—87–112	0.4–14	17–20				
<sup>71</sup> Ga	<sup>79</sup> Y	0.002	0.06	8	2.5–500	100–102	0.2–1.4	6.3–8.3	90–91	0.1–3.6	0.9–3.3
<sup>39</sup> K	<sup>45</sup> Sc	24	40	6	1250–50000	91–97	2.9–3.8	2.8–5.6	85–90	0.3–1.4	1.5–3.6
<sup>139</sup> La	<sup>115</sup> In	0.02	0.05	8	2.5–500	98–101	0.5–0.8	1.9–2.7	95–96	0.4–2.7	1.1–2.8
<sup>7</sup> Li	<sup>45</sup> Sc	0.01	0.06	8	2.5–500	105–107	0.2–1.7	1.6–3.7	99–103	0.2–3.6	0.9–3.7
<sup>24</sup> Mg	<sup>45</sup> Sc	7	10	6	1250–50000	96–97	1.3–3.1	5.4–8.6	87–92	0.9–5.5	3.7–4.7
<sup>55</sup> Mn	<sup>79</sup> Y	0.4	0.5	8	2.5–500	105–106	0.1–1.0	0.8–3.1	96–100	0.4–3.7	1.5–3.1
<sup>98</sup> Mo	<sup>103</sup> Rh	0.05	0.3	8	2.5–500	93–96	1.6–4.2	1.8–3.9	92–94	0.3–0.9	1.1–3.5
<sup>23</sup> Na	<sup>45</sup> Sc	7	25	6	1250–50000	93–97	0.5–2.8	7.6–11	90–92	1.2–3.8	6.1–8.5
<sup>93</sup> Nb	<sup>103</sup> Rh	0.1	0.04	8	2.5–500	99–99	0.1–1.3	5.5–7.4	90–91	0.2–2.4	1.7–4.2
<sup>146</sup> Nd	<sup>115</sup> In	0.01	0.03	8	2.5–500	92–98	0.1–1.8	2.2–3.7	92–94	0.8–1.8	1.1–2.7
<sup>60</sup> Ni	<sup>45</sup> Sc	0.1	0.5	8	2.5–500	97–99	0.3–5.1	1.9–6.0	98–112	1.0–3.7	5.0–12
<sup>31</sup> P	<sup>45</sup> Sc	20	60	4	250–25000	97–101	1.1–3.9	3.3–4.0	88–91	0.2–3.1	2.0–3.8
<sup>208</sup> Pb	<sup>232</sup> Th	0.1	0.3	8	2.5–500	100–102	1.2–2.1	1.3–4.1	96–104	1.1–2.1	2.1–6.5
<sup>141</sup> Pr	<sup>115</sup> In	0.004	0.008	8	2.5–500	95–100	0.5–1.8	0.8–3.2	92–94	0.2–1.9	1.6–2.2
<sup>85</sup> Rb	<sup>79</sup> Y	0.02	0.06	8	2.5–500	98–99	0.3–1.2	5.1–7.0	91–92	0.1–2.6	1.0–3.5
<sup>121</sup> Sb	<sup>115</sup> In	0.006	0.02	8	2.5–500	91–96	1.0–1.4	1.3–2.7	91–92	0.8–3.2	0.5–2.9
<sup>76</sup> Se	<sup>79</sup> Y	0.4	0.6	6	10–500	90–98	0.5–3.2	4.4–9.7	88–90	0.1–2.4	2.0–3.3
<sup>28</sup> Si	<sup>45</sup> Sc	65	270	4	2500–25000	115–117	1.8–13	14–28	89–100	3.9–13	20–27
<sup>118</sup> Sn	<sup>115</sup> In	0.04	0.06	8	2.5–500	96–97	1.5–1.8	1.6–3.3	90–93	0.1–3.1	0.5–2.7
<sup>88</sup> Sr	<sup>79</sup> Y	0.7	1	6	550–27500	97–98	0.1–1.6	5.7–8.0	101–106	0.2–2.0	1.6–3.0
<sup>159</sup> Tb	<sup>115</sup> In	0.0005	0.006	8	2.5–500	96–102	1.4–4.0	2.2–5.0	93–96	1.0–2.3	1.7–4.3
<sup>125</sup> Te	<sup>115</sup> In	0.02	0.03	8	2.5–500	92–94	0.9–2.7	2.8–3.6	90–92	0.3–2.5	0.5–2.9
<sup>49</sup> Ti	<sup>45</sup> Sc	0.2	0.4	8	2.5–500	100–103	0.4–2.5	2.3–2.7	95–98	0.4–2.9	0.7–2.7
<sup>205</sup> Tl	<sup>232</sup> Th	0.001	0.06	8	2.5–500	102–104	0.9–2.7	2.3–4.0	98–105	1.2–1.4	1.9–6.5
<sup>238</sup> U	<sup>232</sup> Th	0.001	0.005	8	2.5–500	96–98	0.4–1.4	1.1–4.0	92–98	0.5–2.5	1.9–5.4
<sup>51</sup> V	<sup>79</sup> Y	0.04	0.08	8	2.5–500	101–103	0.7–1.8	0.6–2.3	97–100	0.5–1.1	1.0–2.8
<sup>182</sup> W	<sup>232</sup> Th	0.2	0.3	8	2.5–500	101–105	0.6–2.5	1.4–4.4	96–99	0.6–1.5	1.8–5.5
<sup>66</sup> Zn	<sup>79</sup> Y	8	20	5	50–1000	101–120	6.7–14	15–16	90–95	3.6–15	5.8–16
<sup>90</sup> Zr	<sup>79</sup> Y	0.07	0.1	8	2.5–500	95–98	0.1–1.3	7.2–8.9	85–92	0.5–1.3	0.9–2.6

<sup>a</sup> IDL, instrumental detection limit.

<sup>b</sup> MDL, method detection limit.

<sup>c</sup> Linearity range: LQL, lower limit of quantification; UQL, upper limit of quantification; N, number of calibration points.

<sup>d</sup> The low and high spike concentrations were for all trace elements 0.005 and 0.02 mg L<sup>-1</sup> and for major elements (B, Ca, K, Mg, Na, P, Si and Sr) 0.2 and 1 mg L<sup>-1</sup>, respectively.

**Table 3**  
Element contents ( $\mu\text{g kg}^{-1}$ ) in extra-virgin olive oils (EVOO) available on the Italian market.

Element	EVOO n = 24				Italian EVOO n = 11				European EVOO n = 13				p-value <sup>a</sup>
	n > MDL (%)	median	min	max	n > MDL (%)	median	min	max	n > MDL (%)	median	min	max	
Ag	0	<0.06	<0.06	<0.06	0	<0.06	<0.06	<0.06	0	<0.06	<0.06	<0.06	–
Al	100	38	22	166	100	38	29	166	100	38	22	65	ns
As	0	<0.3	<0.3	<0.3	0	<0.3	<0.3	<0.3	0	<0.3	<0.3	<0.3	–
B	0	<20	<20	<20	0	<20	<20	<20	0	<20	<20	<20	–
Ba	92	1.2	<0.7	2.2	100	1.1	<0.7	2.1	85	1.4	<0.7	2.2	–
Be	100	<b>0.054</b>	<b>0.019</b>	<b>0.126</b>	100	0.046	0.019	0.097	100	0.058	0.027	0.126	ns
Bi	42	0.1	0.1	0.3	54	0.2	0.1	0.3	38	0.1	0.1	0.3	–
Ca	100	10,164	9661	11,503	100	10,149	9661	11,503	100	10,172	9765	11,489	ns
Cd	100	0.41	0.34	0.79	100	0.40	0.34	0.48	100	0.42	0.35	0.79	ns
Ce	100	0.3	0.1	0.8	100	0.3	0.1	0.4	100	0.3	0.2	0.8	ns
Co	100	0.12	0.06	0.39	100	0.12	0.09	0.23	100	0.11	0.06	0.39	ns
Cr	100	<b>2.9</b>	<b>1.7</b>	<b>7.2</b>	100	3.0	1.7	7.2	100	2.7	2.1	6.4	ns
Cs	100	0.028	0.011	0.072	100	0.029	0.011	0.052	100	0.028	0.011	0.072	ns
Cu	100	<b>3.7</b>	<b>2.8</b>	<b>17.3</b>	100	3.7	2.8	17.3	100	3.7	2.9	6.1	ns
Dy	100	0.026	0.013	0.042	100	0.027	0.013	0.032	100	0.026	0.016	0.042	ns
Fe	75	42	<12	262	54	23	<12	262	92	46	<12	154	ns
Ga	54	0.07	<0.06	0.44	77	0.10	0.03	0.14	38	<0.06	<0.06	0.44	ns
K	8	<40	<40	48	18	<40	<40	48	0	<40	<40	<40	–
La	100	0.15	0.09	0.48	100	0.14	0.09	0.28	100	0.16	0.12	0.48	ns
Li	100	<b>0.17</b>	<b>0.10</b>	<b>0.46</b>	100	0.15	0.10	0.36	100	0.18	0.11	0.46	ns
Mg	100	<b>218</b>	<b>198</b>	<b>431</b>	100	221	203	431	100	217	198	286	ns
Mn	100	3.5	2.9	11.6	100	3.5	2.9	6.3	100	3.5	3.0	11.6	ns
Mo	46	0.3	<0.3	0.9	64	0.3	<0.3	0.5	23	0.3	<0.3	0.9	–
Na	100	<b>182</b>	<b>142</b>	<b>315</b>	100	181	142	311	100	189	153	315	ns
Nb	0	<0.04	<0.04	0.05	0	<0.04	<0.04	<0.04	15	<0.04	<0.04	0.05	–
Nd	100	0.10	0.06	1.60	100	0.10	0.06	1.60	100	0.10	0.07	0.21	ns
Ni	100	4.8	4.2	9.8	100	4.8	4.3	9.8	100	4.8	4.2	8.4	ns
P	100	275	188	2224	100	280	234	2224	100	272	188	347	–
Pb	100	1.6	1.3	5.5	100	1.6	1.4	2.3	100	1.7	1.3	5.5	ns
Pr	100	0.036	0.017	0.364	100	0.038	0.017	0.364	100	0.035	0.024	0.070	ns
Rb	100	0.28	0.20	4.20	100	0.29	0.20	4.20	100	0.27	0.20	0.64	ns
Sb	100	0.11	0.07	0.45	100	0.11	0.07	0.45	100	0.11	0.07	0.44	ns
Se	42	1.4	<0.6	4.7	45	1.3	0.3	3.2	31	1.5	<0.6	4.7	–
Si	0	<270	<270	<270	0	<270	<270	<270	<270	<270	<270	–	–
Sn	100	0.19	0.12	0.85	100	0.18	0.12	0.85	100	0.20	0.12	0.70	–
Sr	100	7.0	5.4	10.0	100	7	6	9	100	7	5	10	ns
Tb	92	0.018	<0.006	0.035	91	0.019	<0.006	0.025	91	0.017	<0.006	0.035	ns
Te	54	0.05	<0.03	0.14	36	0.06	<0.03	0.12	77	0.05	<0.03	0.14	–
Ti	100	1.6	1.1	4.2	100	1.5	1.1	2.1	100	1.7	1.3	4.2	*
Tl	54	0.06	<0.06	0.15	73	0.08	<0.06	0.10	38	<0.06	<0.06	0.15	–
U	100	<b>0.029</b>	<b>0.019</b>	<b>0.156</b>	100	0.027	0.019	0.068	100	0.030	0.019	0.156	ns
V	100	<b>0.53</b>	<b>0.35</b>	<b>1.37</b>	100	<b>0.52</b>	<b>0.35</b>	<b>0.68</b>	100	<b>0.56</b>	<b>0.40</b>	<b>1.37</b>	*
W	100	1.1	0.9	2.4	100	1.1	0.9	1.8	100	1.1	0.9	2.4	ns
Zn	100	445	417	492	100	436	430	492	100	448	417	483	ns
Zr	100	<b>0.2</b>	<b>0.1</b>	<b>1.5</b>	100	<b>0.1</b>	<b>0.1</b>	<b>0.5</b>	100	<b>0.2</b>	<b>0.1</b>	<b>1.5</b>	*

<sup>a</sup> Non-parametric Mann Whitney test was applied: “–” = not determined; “ns” = not significant at  $p > 0.05$ ; “\*” =  $p < 0.05$ ; “\*\*” =  $p < 0.01$ ; “\*\*\*” =  $p < 0.001$ . Numbers in bold in the same row indicate significant differences ( $p < 0.05$ ).

deionized water. Then, the upper oil phase was accurately removed with the aid of a micropipette and 1 mL lower aqueous phase was further diluted (final sample dilution factor of 200x for the Ag, Al, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, Ti, V and Zn and 10x for the B, Ba, Ca, Mg, Na, P and Si determination) with deionized water before the ICP-MS analysis.

The EVOO used for the spike recovery and method validation were obtained from Italian local supermarkets. An aliquot (0.5 g) of each oil sample was directly weighed into a 10 mL polypropylene tube. Then, reagent mixture F (5 mL) was added to each sample before heating in a water bath (95 °C, 40 min). The upper oil phase of digests was accurately removed with the aid of a micropipette and the lower aqueous phase was analyzed by ICP-MS and ICP-OES (for RCC determination) without further dilutions. All samples were analyzed in duplicate. Further details are reported in the following section.

## 2.5. Quality assurance

Method validation was performed by evaluating the basic performance criteria: accuracy, precision, and method detection and quantification limits (MDL and MQL, respectively) under repeatable conditions

(Konieczka, 2007). Both oil certified material and spiked samples were used to evaluate the analytical performance and quality control. Method blanks, duplicates, and spiked samples were prepared along with every batch of digested and extracted samples. Each digestion/extraction batch contained at least three method blanks to monitor contamination. Standard solutions of all elements were prepared for low and high spikes; 0.05 mL of spike solution was added to appropriate tubes before the addition of reagent mixture and final volumes were adjusted accordingly. The low and high spike concentrations used for recovery experiments were for all trace elements 0.005 and 0.02  $\text{mg L}^{-1}$  and major elements (B, Ca, K, Mg, Na, P, Si, and Sr) 0.2 and 1  $\text{mg L}^{-1}$ , respectively (Table 2).

The instrumental detection limit (IDL) and MDL of each element were calculated according to the formula reported in previous work (D'Ilio et al., 2008):  $\text{IDL or MDL} = 3 \text{ SD } C_{\text{spike}} / (I_{\text{spike}} - I)$ , where C is the concentration expressed in  $\mu\text{g L}^{-1}$ , SD is the standard deviation and I is the signal intensity of the element. Six pool method blanks or digested oil samples and six pool method blanks or digested oil samples spiked with 0.005 and 0.2  $\text{mg L}^{-1}$  for trace and major elements, respectively, were prepared and analyzed. The method blanks were used to calculate

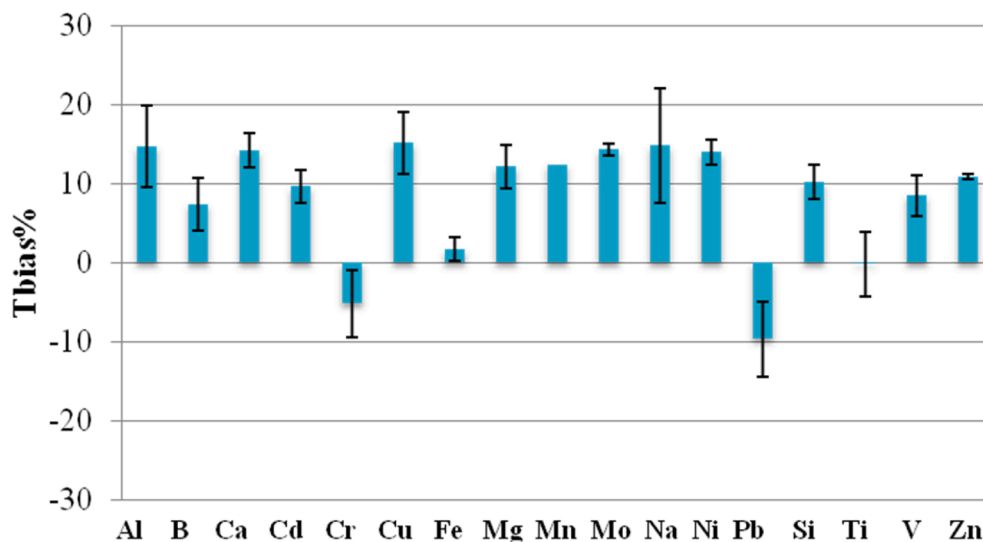


Fig. 1. Trueness bias percentage with standard deviation for the certified elements in oil CRM by WD (5 mL reagent mixture F; 95 °C; 40 min).

IDLs, while digested oil samples were used for the calculation of MDLs (Table 2). Final dilution and weight were taken into account to calculate the IDL and MDL final values. MQLs are defined as the lower and upper quantification limit (LQL and UQL, respectively), which are the lowest and highest points of the calibration curve (Table 2).

## 2.6. Calibration procedure

The calibration ranges were selected according to the expected concentrations of the elements of interest in the analyzed oil samples, and all standard solutions were prepared in the same percentage of acid present in the oil digests. The ICP-MS calibration curves, consisting of concentrations between 0.00025 and 0.05 mg L<sup>-1</sup> and 0.0125 and 5 mg L<sup>-1</sup>, were prepared for all trace and major elements, respectively (Table 2). RCC was determined by ICP-OES using standard solutions of citric acid in the range of 5 to 20 mg L<sup>-1</sup> (Astolfi et al., 2018). The correlation coefficient R<sup>2</sup> obtained for the calibration curves of all elements was at least 0.999, excluding Ca (R<sup>2</sup> = 0.994), by ICP-MS, and the linear concentration range was verified by Mandel fitting test. The influence of instrumental drift for the ICP-MS or ICP-OES analysis was corrected using an internal standard solution of In, Rh, Sc, Th, and Y or only of Y. During the analysis, check standards and blank samples were analyzed every 20 samples, and re-calibration was performed every 100 samples.

## 2.7. Statistical analysis

SPSS Statistics 25 software (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. Non-parametric tests (Kruskal-Wallis and pairwise post-hoc tests) were used to compare different categories of oil samples because of the low and unequal numbers of samples per group and the not always normal distribution (Siegel & Castellán, 1988). A p-value lower than 0.05 was considered statistically significant.

## 3. Results and discussion

Sample treatment procedures were optimized by reducing all reagent volumes, allowing sample preparation and analysis in one 10 mL tube. This prevents sample loss through transfer and reduces sample manipulation and possible contamination from consumables or the atmosphere. The reagent mixtures for oil sample treatment commonly used in the literature are concentrated HNO<sub>3</sub> (Benincasa et al., 2007; Llorent-Martínez et al., 2011a, 2011b), HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> (Bakircioglu et al., 2013)

and HNO<sub>3</sub>/HCl in different ratios (Bakircioglu et al., 2013; Beltrán et al., 2015). In general, HNO<sub>3</sub> is preferred as reagent because it allows the oxidation of almost all organic compounds and causes minor spectral interferences or problems in ICP-MS compared to other reagents (such as HCl). Moreover, treatment with HNO<sub>3</sub> favors Cl removal as nitrosyl chloride and minimizes the polyatomic interferences in the case of Cr, Ni, and As determinations (Cava-Montesinos, Cervera, Pastor, & de la Guardia, 2005). For this reason, the digests obtained with aqua regia were analyzed only with ICP-OES.

### 3.1. Detection and quantification limits (IDL, MDL, and MQL)

Table 2 shows a summary of the IDL, MDL, and MQL for elemental analysis using ICP-MS. The IDL was included for the comparison of instrument capabilities with other instrumentations.

The selected elements in EVOO had concentrations 100% greater than the MDL, except for Ag, As, B, Ba, Bi, Fe, Ga, K, Mo, Nb, Se, Si, Tb, Te, and Tl, whose concentrations were in the range 0–92% greater than the MDL (Table 3). Only the Ca, Cu, Mn, Ni, and Zn levels in the EVOO samples were 100% greater than the QL. For the statistical analyses, we used the original concentrations of all elements, even those with values lower than the LQL, excluding the elements with a percentage of values < MDL greater than 20% (As, B, Bi, Ga, K, Mo, Nb, Se, Si, Te, and Tl), and not completely extracted using the proposed method (Ag, Ba, P, and Sn).

The obtained MDL values for As, Cu, Fe, and Pb were at least 150 times lower than the MRLs established by the IOC for olive and pomace-olive oils, which are 100 µg kg<sup>-1</sup> for As, Cu, and Pb and 3000 µg kg<sup>-1</sup> for Fe (International Olive Council, 2009). In the same way, the maximum values found for Cu (17.3 µg kg<sup>-1</sup>) and Fe (262 µg kg<sup>-1</sup>) in EVOO samples were about four times lower than the recommended maximum concentrations for refined vegetable oils, which are 100 and 1500 µg kg<sup>-1</sup>, respectively (Codex Stan 33–1981). MDLs for other elements were similar to those reported in other methods using ICP-MS as detection technique (Damak et al., 2019; Llorent-Martínez et al., 2011; Savio et al., 2014).

### 3.2. Accuracy and precision

There are not certified reference materials for EVOO. Hence, to validate the sample procedures, we used an oil CRM and recovery experiments over three different EVOO.

The oil CRM, with 21 elements (Ag, Al, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, Pb, Si, Sn, Ti, V, and Zn), was analyzed using both WD

Table 4

Summary of all sample treatment procedures that give a repeatability and trueness bias percentage for the certified elements in oil CRM within 15%.

Element	WD (95 °C)			USE (19 °C)		
	10 min	20 min	40 min	10 min	20 min	40 min
Ag	5 mL A	5 mL A	5 mL A	–	–	–
Al	0.5 mL B; 1 mL B or F; 5 mL A, B, E and F	1 mL A, B and F; 5 mL A or B	0.5 mL A, B, E and F; 1 mL A, B and F; 5 mL A or F	1 mL B, C and F; 5 mL B	0.5 mL B; 1 mL A, B and C, F; 5 mL B or F	0.5 mL A or B; 1 mL A, C and F; 5 mL A or B
B	0.5 mL or 1 mL B and F; 5 mL A, B, E and F	0.5 mL B; 1 mL F; 5 mL A, B, E and F	0.5 mL B, E and F; 1 mL A, B and F; 5 mL A, B, E and F	1 mL B or F; 5 mL B	0.5 mL B; 1 mL A or F; 5 mL A or B	0.5 mL B; 1 mL B, C and F; 5 mL A or B
Ba	–	–	–	–	–	–
Ca	0.5 mL or 1 mL B and F; 5 mL A, B, E and F	0.5 mL B or F; 1 mL A or F; 5 mL A, B, E and F	0.5 mL B or F; 1 mL or 5 mL A, B and F	1 mL F	0.5 mL B; 1 mL A or F; 5 mL A, B and E	0.5 mL or 1 mL B and F; 5 mL B
Cd	0.5 mL B; 1 mL B or F; 5 mL A, B, E and F	0.5 mL B and F or 1 mL F; 5 mL A, B, E and F	0.5 or 1 mL B and F; 5 mL A, B, E and F	1 mL B or F; 5 mL B	0.5 mL A or B; 1 mL A, B and F; 5 mL B	0.5 mL A or B; 1 mL B or F, 5 mL A or B
Cr	5 mL A, B and E	5 mL A, B, E and F	1 mL B; 5 mL A, B, E and F	–	–	–
Cu	0.5 or 1 mL B and F; 5 mL A, B, E and F	0.5 mL B or F; 1 mL B, E and F; 5 mL A, B, E and F	0.5 mL or 1 mL B and F; 5 mL A, B, E and F	1 mL A, B, C and F; 5 mL B	0.5 mL B; 1 mL A, B, C and F; 5 mL B	0.5 mL or 1 mL B and F; 5 mL A and B
Fe	0.5 or 1 mL B; 5 mL A or B	0.5 or 1 mL B; 5 mL A, B and F	0.5 mL or 1 mL B; 5 mL A, B and F	5 mL B	5 mL B	0.5 mL B; 1 mL or 5 mL A and B
Mg	0.5 or 1 mL B and F; 5 mL A, B, E and F	0.5 mL B or F; 1 mL B; 5 mL A, B, E and F	0.5 mL or 1 mL B; 5 mL A, B, E and F	1 mL B or F; 5 mL B	0.5 mL A or B; 1 mL B or F; 5 mL B	0.5 mL or 1 mL A, B and F; 5 mL A and B
Mn	0.5 mL or 1 mL B and F; 5 mL A, B, E and F	0.5 mL B or F; 1 mL A, B and F; 5 mL A, B, E and F	0.5 mL B or F; 1 mL A, B and F; 5 mL A, B, E and F	1 mL B or F; 5 mL B	0.5 mL B; 1 mL A, B and F; 5 mL B	0.5 mL or 1 mL B or F; 5 mL A or B
Mo	0.5 mL B or E; 1 mL B, E and F; 5 mL A, B, E and F	0.5 mL B; 1 mL E or F; 5 mL A, B, E and F	0.5 mL B or E; 1 mL B, E and F; 5 mL A, B, E and F	1 mL B or F; 5 mL B	0.5 mL B; 1 mL F; 5 mL A or B	0.5 mL B or F; 1 mL F; 5 mL A or B
Na	0.5 mL F; 1 mL B or F; 5 mL A	0.5 mL F; 5 mL B	0.5 mL F; 1 mL B or F; 5 mL A, B and F	1 mL B or F; 5 mL B	1 mL A, B and F	0.5 mL B or F; 1 mL A, B and F; 5 mL B
Ni	0.5 mL or 1 mL B and F; 5 mL A, E and F	0.5 mL or 1 mL B and F; 5 mL A, B, E and F	0.5 mL or 1 mL B and F; 5 mL A, B, E and F	1 mL B or F; 5 mL B	0.5 mL A or B; 1 mL A, B and F; 5 mL B	0.5 mL A, B and F; 1 mL B or F; 5 mL A or B
P	–	–	–	–	–	–
Pb	5 mL A or B	1 mL A; 5 mL A or B	1 mL A or B; 5 mL B or F	–	5 mL A	1 mL C or F; 5

Table 4 (continued)

Element	WD (95 °C)			USE (19 °C)		
	10 min	20 min	40 min	10 min	20 min	40 min
Si	5 mL B, C, D, E and F	5 mL A, B, E and F	5 mL A, B, C, E and F	5 mL A, B, C, D, E and F	5 mL A, B, C, E and F	5 mL A, B, C, D, E and F
Sn	5 mL A	5 mL A	5 mL A	–	–	–
Ti	5 mL A or B	5 mL A, B and F	5 mL A, B and F	–	–	1 mL F; 5 mL A
V	0.5 mL B; 1 mL B, E and F; 5 mL A, B, E and F	0.5 mL B; 1 mL A, E and F; 5 mL A, B, E and F	0.5 mL B, E and F; 1 mL A, B and F; 5 mL A, B, E and F	1 mL B or F; 5 mL B	0.5 mL B; 1 mL C or F; 5 mL A or B	0.5 mL B or F; 1 mL C or F; 5 mL A or B
Zn	0.5 or 1 mL B and F; 5 mL A, B, E and F	0.5 mL B or F; 1 mL F; 5 mL A, B, E and F	0.5 mL or 1 mL B and F; 5 mL A, B, E and F	1 mL B or F; 5 mL B	0.5 or 5 mL B; 1 mL F	0.5 mL B or F; 1 mL C or F; 5 mL A or B

and USE methods. Various oxidizing reagents [A = aqua regia; B = HNO<sub>3</sub>; C = HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, 2:1 v/v; D = 5% (v/v) HNO<sub>3</sub>; E = H<sub>2</sub>O<sub>2</sub> and F = 10% (v/v) HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, 2:1 v/v] were prepared and used in both USE and WD methods with different times (10, 20 and 40 min). All results of the trueness bias percentage (Tbias%) and repeatability (CVR%) for the certified elements in oil CRM are shown in Tables S3-S11 and Fig. 1, while a summary of sample treatment procedures that gives a Tbias and CVR% within 15% is shown in Table 4. Only P fell outside this range with Tbias% < -91.3%. Acceptable results for Ag and Sn were only obtained using WD and reagent mixture A (5 mL; 10, 20, or 40 min), and for Ba using WD and reagent mixture B (5 mL; 40 min). The results show better accuracy and precision values were obtained as reagent volume mixture, acidity, temperature, and treatment duration increase. However, for some elements (Al, B, Ca, Cd, Cr, Cu, Mg, Mn, Mo, Ni, Si, V, and Zn), results of Tbias and CVR% within 15% were also obtained only by using H<sub>2</sub>O<sub>2</sub> (reagent mixture E). Considering these results, the low concentrations in real samples and the not required dilution of solution F prior to ICP-MS analysis, we chose to study the recoveries on real samples using the WD method with 5 mL reagent mixture F for 40 min. The digest obtained from solution F does not require dilution before instrumental analysis. It has an acidity lower than 5% (maximum acidity possible to perform the ICP-MS analysis, as suggested by the instrument's user manual). Higher sample acidity could increase polyatomic interferences and cause damage to the interface of the instrument. In addition to reducing both reagent volumes and dilutions, this approach allows preparation and instrumental analysis in a single tube, preventing sample loss and minimizing sample handling.

Recovery experiments were performed in three EVOO at two concentration levels [for all trace elements at 0.005 and 0.02 mg L<sup>-1</sup>, and major elements (B, Ca, K, Mg, Na, P, Si and Sr) at 0.2 and 1 mg L<sup>-1</sup>, respectively]. The recoveries, shown in Tables 2 and S12-S14, fell within 20% of the expected value and many of the elements in all the analyzed EVOO (Ag, Al, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Dy, Ga, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sn, Sr, Tb, Te, Ti, Tl, U, V, and W) recovering within 10%. The obtained recoveries confirmed that no significant element losses occurred during the WD procedure. Intra-day and inter-day replicate analyses with the proposed method had good CVR% and reproducibility (CVR%), lower than 15 and 20%, respectively, excluding CVR% of Si at higher spike level (Tables 2 and S12-S14). Good results obtained by the proposed method indicate that this oil sample treatment procedure is satisfactory to release internally bound elements. Moreover, this method was optimized by reducing acidity, volumes, and final

dilution, thus allowing sample preparation and analysis in only one vessel. This prevented sample loss, minimized volume transfer errors and reduced the risk of contamination from consumables or the atmosphere.

### 3.3. Application of the WD method

The proposed WD method (5 mL reagent mixture F; 95 °C; 40 min) was applied to the elemental determination of 24 EVOO samples. Each sample was independently digested and analyzed in duplicate. The concentrations obtained for each oil sample are shown in Table 3. It was not possible to compare the obtained data for Ag, Ba, P and Sn, because these elements were not completely extracted. In general, the presence of elements (such as As, Cu, Fe, and Pb) is undesirable because they can be toxic to customers' health and facilitate oil oxidative degradation, decreasing shelf life. In all cases, the observed concentrations of As, Cu, Fe, and Pb were lower than the MRLs allowed (International Olive Council, 2009) and the maximum levels legislated (Codex Stan 33–1981), confirming the excellent quality of EVOO. Variation in elements was noted across different samples, thus indicating the method's usefulness for elemental fingerprinting. The contents of Ti, V, and Zr in Italian EVOO (1.5, 0.52, and 0.1  $\mu\text{g kg}^{-1}$ , respectively) were significantly lower than in European EVOO (1.7, 0.56, and 0.2  $\mu\text{g kg}^{-1}$ , respectively). Unfortunately, to our knowledge, only a few studies report Ti, V and Zr concentrations in EVOO samples (Beltrán et al., 2015; Llorent-Martínez et al., 2011; Pošćić et al., 2019) and none in Italian EVOO. The concentrations of V, and Zr found in Italian EVOO of the present study were lower than those reported in the olive oils from Spain ( $V = 13.8\text{--}18.4 \mu\text{g kg}^{-1}$ ; and  $Zr = 3.1\text{--}4.3 \mu\text{g kg}^{-1}$ ) by Beltrán et al. (2015).

Our results agreed the most with those from studies on Italian olive oils (Benincasa et al., 2007; Camin et al., 2010) and Spanish olive oils (Cabrera-Vique et al., 2012; Llorent-Martínez et al., 2011a, 2011b) but for some elements differed from other results in Argentine (Savio et al., 2014) and Tunisian olive oils (Damak et al., 2019). The concentrations of Na found in this study were in the range of 142–315  $\mu\text{g kg}^{-1}$ , which are similar to the median value that was found in Italian olive oils (49–280  $\mu\text{g kg}^{-1}$ ) as reported by Camin et al. (2010), but 20 times lower than the levels reported in Tunisian olive oils (Damak et al., 2019). The concentrations of Cu and Sb measured in the present study ranged between 2.8 and 17.3  $\mu\text{g kg}^{-1}$ , and 0.07 and 0.45  $\mu\text{g kg}^{-1}$ , respectively. These values are much lower than those previously reported in Argentine olive oils ( $\text{Cu} = 260\text{--}1100 \mu\text{g kg}^{-1}$ ; and  $\text{Sb} = 1330\text{--}2030 \mu\text{g kg}^{-1}$ ) (Savio et al., 2014), and are in a similar range in the olive oils from Spain ( $\text{Cu} = 3.35\text{--}66.47 \mu\text{g kg}^{-1}$ ) (Cabrera-Vique et al., 2012), and Italy ( $\text{Sb} = 0.194\text{--}0.411 \mu\text{g kg}^{-1}$ ) (Benincasa et al., 2007).

## 4. Conclusion

Sample preparation is a critical stage in the trace element analysis in edible oil samples. Methods requiring a sample treatment to destroy the organic matrix involve oxidations and the risk of sample contamination. Here we reported a rapid analytical method for the determination of 45 elements in EVOO by ICP-MS, useful for routine and control quality analyses as well as for applications such as geographical fingerprinting. Sample preparation of EVOO (0.5 g) was carried out by WD at 95 °C in 5 mL of mixture F for 40 min. The final digest does not require dilution before ICP-MS analysis. The proposed method is easy to apply and advantageous compared to other approaches used. It allows for sample preparation and instrumental analysis in a single tube, preventing sample loss, minimizing contamination, and reducing both reagent volumes and dilutions requests. This method showed good accuracy and precision for all the analyzed elements, excluding Ag, Ba, P, and Sn.

This study is a valuable aid in choosing not only the most appropriate oil pre-treatment method but also the most suitable reagent mixture for the determination of each selected element.

## CRedit authorship contribution statement

**Maria Luisa Astolfi:** Conceptualization, Validation, Formal analysis, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision. **Elisabetta Marconi:** Investigation, Visualization. **Giulia Vitiello:** Investigation, Writing - original draft. **Lorenzo Mas-simi:** Writing - original draft.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.130027>.

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