



ARCHIVIO ISTITUZIONALE DELLA RICERCA

Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

New polluting metals. Quantification in herbal medicines by voltammetric and spectroscopic analytical methods

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

New polluting metals. Quantification in herbal medicines by voltammetric and spectroscopic analytical methods / Melucci D.; Locatelli M.; Casolari S.; Locatelli C.. - In: JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS. - ISSN 0731-7085. - STAMPA. - 211:(2022), pp. 114599.1-114599.7. [10.1016/j.jpba.2022.114599]

This version is available at: <https://hdl.handle.net/11585/879185> since: 2022-03-22

Published:

DOI: <http://doi.org/10.1016/j.jpba.2022.114599>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

(Article begins on next page)

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

This is the final peer-reviewed accepted manuscript of:

Melucci, D., Locatelli, M., Casolari, S., Locatelli, C., 2022. New polluting metals. Quantification in herbal medicines by voltammetric and spectroscopic analytical methods. Journal of Pharmaceutical and Biomedical Analysis 211, 114599.

The final published version is available online at:
<https://doi.org/10.1016/j.jpba.2022.114599>.

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

18 **Abstract**

19 A new application of voltammetric techniques in stripping mode to the quantitative determination of
20 metals belonging to the platinum group (PGM) in herbal medicines, at the ultra-trace level, is
21 reported. Pd (II), Pt (II) and Rh (III) are determined by means of square-wave adsorption voltammetry
22 (SWAdSV); Os (VIII) and Ru (III) are determined through catalytic square-wave voltammetry
23 (SWCV); Ir (III) is determined through the application of square-wave catalytic adsorption
24 voltammetry (SWAdCSV). In all the applied methods, a conventional three-electrode voltammetric
25 cell is employed, which sees the presence of a suspended mercury-drop electrode (HMDE) or a glassy
26 carbon electrode (GCE) as working electrodes for the determination of Ir (III). The auxiliary electrode
27 was a platinum electrode, and an $\text{Ag}|\text{AgCl}|\text{KCl}_{\text{satd.}}$ electrode was employed as reference electrode.
28 Validation of the analytical procedure here proposed has been achieved using reference standards:
29 NIST-SRM 1570a (Spinach Leaves) and NIST-SRM 1573a (Tomato Leaves), both added with pure
30 metal standards, obtaining more than good precision values and better than the limits set for the
31 validation of quantitative methods. Following the verification of the validity of the procedure
32 indicated here, commercially available herbal medicines, based on *Eucalyptus globulus*,
33 *Harpagophytum procumbens* DC and *Taraxacum officinale weber*, were analyzed. Standard samples
34 were also analyzed by atomic absorption spectroscopy in order to have a reference technique for
35 validating the entire procedure.

36

37

38 **Keywords:** PGMs, Voltammetry, Spectroscopy, Herbal Medicines.

39

40 **1. INTRODUCTION**

41 In recent years, the Scientific Community has paid increasing attention to metals belonging to
42 the platinum group (PGM), especially in the environmental field and following the increase in their
43 concentration in this type of matrices. This effect is attributable to the use of these metals in various
44 anthropic sectors such as the production of industrial catalysts, anticancer drugs, jewelry, and use of
45 autocatalytic converters. This last element, especially linked to the incorrect "stop and go" use of
46 these converters, with consequent deterioration and abrasion, implies a significant release into the
47 environment of airborne particulate matter characterized by high content of PGM and is currently the
48 most relevant source of contamination by PGM. The increase has been all the greater since the
49 transition from platinum, palladium and rhodium-based catalysts to the increasing use of iridium,
50 ruthenium and, above all, osmium [1].

51 The main problem observed with PGMs is their persistence in the environment, with serious
52 damage to food safety. This is above all linked to the fact that they have a tendency to
53 bioaccumulation in all environmental matrices (plants, terrestrial and aquatic organisms) thus
54 representing a way of entry into the food chain. Human beings, as the final element of this supply
55 chain, therefore represent the subjects most exposed and at risk, especially in light of the toxicity of
56 PGMs in their various bioavailable forms [2]. What has been observed so far certainly finds reason
57 to exist in the normal food chain, but the growing use of food supplements entails the need to monitor
58 these PGMs also in these products, even if currently the Scientific Community does not yet consider
59 them in this sense.

60 Food supplements, encompassing a wide range of products ranging from vitamins and
61 minerals to herbal agents (remedies and medicines based on herbs), various extracts and enzymes,
62 require constant evaluation and quality control to ensure that toxic levels fall within acceptable limits.
63 In this context, medicinal herbs are certainly a significant component of the market, if not the
64 dominant one. Herbal medicines are currently referred to as "*products of plant origin used for*

65 *medicinal and/or nutritional purposes"*, encompassing a large range of products, from herbal teas to
66 products for nutritional-care and body-care.

67 Despite herbal medicines can be considered not dangerous to health, being derived from
68 natural products, unfortunately there is currently not much scientific evidence to support this general
69 belief. While synthetic products can be purified and stabilized in their formulations, with a defined
70 dosage to obtain the therapeutic effect and minimizing the adverse effects, herbal medicines are
71 difficult to standardize due to raw extracts containing different active ingredients (phytoextract).
72 This is above all linked to the fact that different pharmacologically active ingredients are present in
73 the phytoextract, often similar to synthetic compounds but potentially showing a greater number of
74 adverse effects [1, 3-4].

75 Another element not to be overlooked is that, to date, there is no real regulation for the
76 production of herbal medicines, combined with the fact that they can be purchased without a
77 prescription. In light of these regulatory deficiencies, international organizations like WHO (World
78 Health Organization and FDA (US Food and Drug Administration) simply recommend to check for
79 the quantity of toxic heavy metals in medicinal plants, starting from raw materials. Only for some of
80 these metals the upper-threshold admissible limits are defined: 1.0 ppm for mercury; 10.0 ppm for
81 lead; 0.3 ppm for cadmium; 10.0 ppm for arsenic [5-6]. For all other metals, a real decision has not
82 been evaluated and/or taken, yet; probably this happens because, in many cases, these metals are
83 considered micronutrients and consequently their potential harmfulness is not adequately taken into
84 account when they are assumed in large quantities.

85 In the analysis of metals, the most widely used instrumental technique is certainly the
86 electrothermal atomic absorption spectroscopy (ETAAS) [7-9], especially in the configuration that
87 envisages the graphite furnace (GFAAS) as an atomization device. In the literature there are some
88 works [10, 11] in which the herbal medicine fingerprint is obtained using this technique, which can
89 then be processed using chemometric approaches to investigate their origin [12], or the possible
90 evaluation of fraud [13].

91 In recent years, a line of research has been expanded in our laboratories that includes
92 investigating new approaches to quantitatively determine PGMs in environmental and food samples
93 [14-30] through the application of such spectroscopic techniques, and only recently to quantify toxic
94 metals in matrices characterizing such categories of samples [8 and bibliography, 31 and
95 bibliography].

96 In this context, especially considering the lack of studies relating to the voltammetric
97 determination of PGMs in herbal medicines, we wanted to develop a procedure to meet the needs
98 related to the quality control and characterization of these matrices. This paper presents studies and
99 results related to the following voltammetric techniques: square wave adsorbent stripping
100 voltammetry (SWAdSV) [Pd (II), Pt (II), Rh (III)], square-wave catalytic voltammetry (SWCV) [Ru
101 (III), Os (VIII)] and square-wave catalytic adsorbent stripping voltammetry with (SWAdCSV).

102

103 **2. EXPERIMENTAL**

104 *2.1 Instrumentation*

105 The acquisition of voltammograms was performed by a Multipolarograph AMEL Mod. 433
106 (Milan, Italy). The measuring cells (three-electrode set-up) is structured in the case of iridium with a
107 working electrode consisting in a glassy-carbon electrode (GCE, AMEL, Milan; 7.065-mm²surface
108 area). For all the other PGMs herein considered, a stationary HMDE was used. In all cases,
109 Ag|AgCl|KCl_{satd.} was the reference electrode and a platinum-wire electrode was the auxiliary
110 electrode. The Teflon voltammetric cell was rinsed many times before carrying out measurements, in
111 order to prevent contamination. This procedure was performed as follow: *i*) a first rinsing with supra-
112 pure HNO₃ 69%_{w/w}, diluted 1:1 with water obtained by a Milli-Q deionizing system (Millipore,
113 Darmstadt, Germany); *ii*) the cell was rinsed many times using Milli-Q water.

114 Disposable plastic tips were used for the standard additions. The solutions were previously
115 thermostated at 20.0±0.5°C and treated by pure N₂ for 5 min to remove oxygen. During the
116 measurements, solutions were kept under nitrogen. The magnetic stirring (with Teflon-coated

117 magnetic stirring bar) was constantly maintained during the purge step. For the analyses here
118 presented, the supporting electrolytes and the voltammetric parameters are reported in Table 1 and
119 Table 2.

120

121 **Table 1.** Instrumental details for Pd(II), Pt(II), Rh(III) by SWAdSV and Pb(II) by SWASV.
122 Supporting electrolytes: HCl 0.15 mol L⁻¹ + dimethylglyoxime 1.9·10⁻⁴ mol L⁻¹ [Pd(II)] and HCl 0.15
123 mol L⁻¹ + dimethylglyoxime 1.9·10⁻⁴ mol L⁻¹ + formaldehyde 0.65 mmol L⁻¹ + hydrazine (formazone
124 complex) 1.25 mmol L⁻¹ in HCl 0.15 mol L⁻¹ [Pt(II) and Rh(III)].

125

126 **Table 2.** Instrumental parameters for Ru(III) and Os(VIII) by SWCV, and Ir(III) by SWAdCSV.
127 Supporting electrolytes: acetate buffer 0.5 mol L⁻¹ pH 4.9 + NaBrO₃ 7.3·10⁻² mol L⁻¹ [Os(VIII),
128 Ru(III)], and acetate buffer 0.5 mol L⁻¹ pH 4.9 + NaBrO₃ 7.3·10⁻² mol L⁻¹ + cetyltrimethylammonium
129 bromide 4.9·10⁻⁵ mol L⁻¹ + KCl 0.25 mol L⁻¹ [Ir (III)].

130

131 An Atomic Absorption Spectrometer by Perkin-Elmer (Mod. A-Analyst 100 with deuterium
132 background-corrector, Autosampler AS-72 and graphite furnace model HGA 800), was used for the
133 acquisition of ET-AAS measurements. The light sources were Lumina hollow-cathode lamps for
134 single-element measurements (Perkin-Elmer, USA), while the steps of ashing and atomization
135 (different from element to element) were set up as reported in the literature [32]. Table 3 reports the
136 instrumental parameters, optimized in the present work.

137

138 **Table 3.** Instrumental details for Pd(II), Pt(II), Ir(III), Os(VIII), Rh(III) and Ru(III) by GF-AAS.
139 Injected volume: 20 μL. Argon flow: 60 mL min⁻¹ only in the atomization step, 300 mL min⁻¹ in all
140 other steps. For all the elements no matrix modifier is employed.

141

142 2.2 Reagents and Reference Solutions

143 Chemicals at suprapure-grade (Merck, Darmstadt, Germany) were used in this work, while a
144 Milli-Q system was used to obtain demineralized water. Reference standard solutions were obtained
145 starting from acidic standard solutions: Ir(III), Pd(II), Pt(II), Rh(III), Ru(III) (1000 mg L⁻¹, Merck,
146 Darmstadt, Germany). Osmium tetroxide solution in water (4% w/w, Sigma-Aldrich, Darmstadt,
147 Germany) was used as starting standard solution. In order to optimize the instrumental parameters,

148 two reference materials were used as standard: NIST-SRM 1570a (Spinach Leaves) and NIST-SRM
149 1573a (Tomato Leaves) (National Institute of Standards and Technology, Gaithersburg, MD, USA).

150

151 2.3 Sample Preparation

152 The standard reference materials (NIST-SRM 1570a and NIST-SRM 1573a) and the herbal
153 medicines (*Eucalyptus globulus*, *Harpagophytum procumbens* DC and *Taraxacum officinale* Weber)
154 were solubilized by an acidic attack using a mixture of HCl, HNO₃ and H₂SO₄.

155 The vegetables were accurately weighed (order of magnitude: 1 g), and put in a 25-mL Pyrex-
156 tube for digestion, and a solution containing 4 mL HNO₃ 69% w/w, 4 mL HCl 37% w/w and 5 mL H₂SO₄
157 96% w/w was added. Then a Vigreux column condenser was connected to the tube, and the tube was
158 inserted into a cold block digester (appositely homemade); finally, the temperature was progressively
159 raised up to 150°C and maintained at this final temperature for 2 h to achieve the mineralization.
160 After this step, the content was dried, cooled, and dissolved in Milli-Q water (25 mL) or in 25 mL of
161 the supporting electrolyte.

162

163 2.4 Voltammetric procedure for Pd(II)-Pt(II)-Rh(III)

164 The voltammetric procedure for Pd(II)-Pt(II)-Rh(III) consists in two steps: *i*) 10-mL sample
165 aliquot of HCl 0.15 mol L⁻¹ + dimethylglyoxime (DMG) 1.9·10⁻⁴ mol L⁻¹ aqueous reference solution
166 or solutions obtained in the mineralization step were transferred into the voltammetric cell; de-
167 aeration was achieved by bubbling water-saturated pure N₂ for 5 min. The determination of Pd(II)
168 was carried out by SWAdSV; *ii*) 0.5 mL of formaldehyde 1.37·10⁻² mol L⁻¹ + hydrazine (formazone
169 complex) 2.63·10⁻² mol L⁻¹ in HCl 0.15 mol L⁻¹ solution (final concentration: formaldehyde = 0.65
170 mmol L⁻¹ and hydrazine = 1.25 mmol L⁻¹) were added. The determination of Pt(II) and Rh(III) was
171 carried out by SWAdSV.

172

173 2.5 Possible interference from Pb(II) and Zn(II) in quantifying Pd(II) and Pt(II) by voltammetry

174 In the experimental conditions applied herein for the Pd(II), Pt(II) and Rh(III) determination,
175 also Pb(II) and Zn(II) show voltammetric peaks (-0.485 and -0.877 V vs. Ag|AgCl|KCl_{satd.},
176 respectively). Hence, there is a possible interference with Pd (II) and Pt (II) (-0.343 and -1.027 V
177 vs. Ag|AgCl|KCl_{satd.}, respectively), involving in two possible interference cases: Pd(II)-Pb(II) and
178 Pt(II)-Zn(II). Figure 1 reports the voltammetric peak due to Pb (II), which shows very low
179 reversibility degree in the employed supporting electrolyte. However, it is worth pointing out that this
180 element, when present at very high concentration ratios ($c_{\text{Pb(II)}} : c_{\text{Pd(II)}} > 650 \mu\text{g g}^{-1}$), certainly could
181 be a possible interfering species in the Pd(II) voltammetric determination.

182

183 **Figure 1.** Square-wave adsorptive stripping voltammogram: determination of Pd(II) in the presence
184 of Pb(II) in *Taraxacum officinale weber*. Supporting electrolyte: HCl 0.15 mol L⁻¹ + DMG 1.9·10⁻⁴
185 mol L⁻¹. Peak 1 corresponds to Pd(II); peak 2 corresponds to Pb(II).
186 Experimental conditions: see Table 1
187

188 Similarly, Zn(II) may interfere during Pt(II) quantification, showing a voltammetric peak at –
189 0.877 V vs. Ag|AgCl|KCl_{satd.} (Figure 2). Again, the Zn(II) electrode process shows a low
190 reversibility degree in the employed supporting electrolyte, and the interference can be observed only in
191 the case of very high concentration ratios ($c_{\text{Zn(II)}} : c_{\text{Pt(II)}} > 750 \mu\text{g g}^{-1}$).

192

193 **Figure 2.** Square-wave adsorptive stripping voltammogram: determination of Pt(II) and Rh(III) in
194 the presence of Zn(II) in *Taraxacum officinale weber*. Supporting electrolyte: HCl 0.15 mol L⁻¹ +
195 DMG 1.9·10⁻⁴ mol L⁻¹ + formaldehyde 0.65 mmol L⁻¹ + hydrazine (formazone complex in 0.15 mol
196 L⁻¹ HCl) 1.25 mmol L⁻¹. Peak 1 corresponds to Zn(II), peak 2 corresponds to Pt(II), peak 3
197 corresponds to Rh(III). Experimental conditions: Table 1.
198

199 2.6 Voltammetric procedure for Ir(III), Os(VIII) and Ru(III)

200 The voltammetric procedure for Ir(III)-Os(VIII)-Ru(III) is carried out two steps:

201 i) 10-mL sample aliquots of acetate buffer 0.5 mol L⁻¹ pH 4.9 + NaBrO₃ 7.3·10⁻² mol L⁻¹ aqueous
202 reference solution, or of solutions obtained in the mineralisation step containing acetate buffer 0.5
203 mol L⁻¹ pH 4.9 + NaBrO₃ 7.3·10⁻² mol L⁻¹, were transferred into the voltammetric cell; de-aeration

204 was kept for 5 min by bubbling water-saturated pure N₂. The determination of Os(VIII) and Ru(III)
205 was performed by SWCV with HMDE electrode

206 ii) HMDE electrode was replaced with GCE electrode, in the same voltammetric cell; then, 1 mL of
207 cetyltrimethylammonium bromide (CTAB) $5.98 \cdot 10^{-4}$ mol L⁻¹ (final CTAB concentration $4.9 \cdot 10^{-5}$ mol
208 L⁻¹) and 1 mL of KCl 3.0 mol L⁻¹ (final KCl concentration 0.25 mol L⁻¹) were added for the
209 quantification of Ir(III) by SWAdCSV.

210

211 2.7 Voltammetric interference from Cu(II) and Pb(II) in the Os(VIII) and Ru(III) determination

212 Cu(II) and Pb(II) (-0.169 ± 0.015 and -0.327 ± 0.010 V vs. Ag|AgCl|KCl_{satd.}, respectively) in
213 acetate buffer 0.5 mol L⁻¹ pH 4.9 + NaBrO₃ $7.3 \cdot 10^{-2}$ mol L⁻¹ supporting electrolyte could interfere
214 strongly with the determination of Os(VIII) and Ru(III) (-0.243 ± 0.010 and -0.023 ± 0.015 V vs.
215 Ag|AgCl|KCl_{satd.}, respectively). This phenomenon, reported in Figure 3a, is decidedly important
216 also related to the fact that Cu(II) and Pb(II), in general, are always present in all real matrices, in
217 particular the environmental ones, even if at low-concentration level. This drawback can be avoided
218 using the well-known ability of Ethylene Diamine Tetraacetic Acid (EDTA) to shift peaks toward
219 cathodic potentials if added to the voltammetric cell. In this work, Cu(II) and Pb(II) interfering peaks
220 were shifted by adding EDTA di-sodium salt, specifically: 200 μL EDTA-Na₂ $9.6 \cdot 10^{-3}$ mol L⁻¹ were
221 added to a 10-mL sample aliquot, obtaining $1.88 \cdot 10^{-4}$ mol L⁻¹ as final EDTA-Na₂ concentration.
222 Indeed, the EDTA-Na₂ presence results in a shift of potential values in the cathodic direction, either
223 for Os(VIII) and Ru(III) or Cu(II) and Pb(II) [Os(VIII): -0.096 ± 0.015 ; Ru(III): -0.529 ± 0.015 ; Cu(II):
224 -0.301 ± 0.015 ; Pb(II): -0.751 ± 0.010 V vs. Ag|AgCl|KCl_{satd.}]. Under these conditions, the final peak
225 position of the four elements makes it possible to achieve complete resolution (see Figure 3b), and
226 consequently the quantitative determination becomes possible.

227

228 **Figure 3.** Square-wave catalytic voltammogram: Os(VIII) and Ru(III) in presence of Cu(II) and
229 Pb(II) in *Taraxacum officinale weber*. Supporting electrolyte: acetate buffer 0.5 mol L⁻¹ pH 4.9 +
230 NaBrO₃ $7.3 \cdot 10^{-2}$ mol L⁻¹ in absence (a) and presence (b) of EDTA-Na₂. Peak 1 corresponds to

231 Os(VIII), peak 2 corresponds to Cu(II), peak 3 corresponds to Ru(III), peak 4 corresponds to Pb(II).
232 Experimental conditions: see Table 2.
233

234 As for Ir(III) (see Figure 4), EDTA-Na₂ seems to slightly change the position [Ir(III) peak
235 potential in presence of EDTA-Na₂ 1.88 · 10⁻⁴ mol L⁻¹: +0.623 ± 0.015 V vs. Ag|AgCl|KCl_{satd.}; Ir(III)
236 peak potential without EDTA-Na₂: +0.609 ± 0.010 V vs. Ag|AgCl|KCl_{satd.}], keeping the peak height
237 practically unchanged. Following these experimental observations, several elements can be smoothly
238 determined by the procedure suggested here.

239

240 **Figure 4.** Square-wave adsorptive catalytic stripping voltammogram: determination of Ir(III) in
241 *Taraxacum officinale weber*. Supporting electrolyte: acetate buffer 0.5 mol L⁻¹ pH 4.9 + NaBrO₃
242 7.3 · 10⁻² mol L⁻¹ + CTAB 4.9 · 10⁻⁵ mol L⁻¹ + KCl 0.25 mol L⁻¹. Experimental conditions: see Table 2.
243

244 3. RESULTS AND DISCUSSION

245 3.1 Aqueous Reference Solutions

246 The calculation of limits of detection (LOD) (Table 4) was performed according to IUPAC
247 by the “3 σ approach”, where σ is the observed standard deviation. LODs were calculated in the
248 aqueous reference solution and in the digestates obtained from standard reference materials. LODs
249 were calculated as $(K s_{y/x})/b$ [33-34] ($K=3$), where $s_{y/x}$ is the standard deviation of regression and b is
250 the slope of the analytical calibration function. When voltammetry was used, LODs were obtained
251 directly in the real matrices (Table 4); in fact, the standard addition method was applied to create the
252 analytical calibration model. For each analyte, linearity was explored through regression models of
253 voltammetric signal vs. metal concentration in aqueous reference solution with the instrumental
254 details reported in Tables 1 and 2. The linearity of the model was demonstrated in the range from
255 LOQs up to 10.0 μg L⁻¹. Determination coefficients R^2 were always higher than 0.9989.

256

257 **Table 4.** Limits of detection of Ir(III), Os(VIII), Pd(II), Pt(II), Rh(III), Ru(III), in aqueous
258 reference solutions and in solutions from reference materials. Experimental values are calculated as
259 the mean of 5 independent determinations. The confidence level was 95 %.

260
261

3.2 Quality control and quality assessment

262 Standard reference materials of Spinach Leaves and Tomato Leaves were analysed by means
263 of voltammetric and spectroscopic method for validating the whole method through the evaluation of
264 its accuracy (Table 5). Furthermore, the repeatability was evaluated as relative standard deviation
265 ($s_r\%$) [33-34], from 5 independent measurements, and always resulted better than 6%. Trueness was
266 evaluated through the relative error ($e\%$), and resulted to be on the order of 5-7%. These experimental
267 values observed for the method correspond to satisfactory accuracy.

268

269 **Table 5.** Accuracy of the analytical procedure. Experimental values are calculated as the mean of 5
270 independent determinations. The confidence level was 95%. The spiked-sample concentration was
271 always 79.5 ng g⁻¹ (the addition to the Standard Reference Materials took place at the beginning of
272 digestion).

273
274
275

3.3 Practical applications

276 Since the quantification of PGMs in reference materials gave good accuracy, the methods
277 were applied to real samples: herbal medicines sold on the local market, and based on *Eucalyptus*
278 *globulus*, *Harpagophytum procumbens DC* and *Taraxacum officinale weber*. The section “Sample
279 Preparation” describes how samples were treated, after a preliminary step of powdering, by
280 lyophilisation and dried at 80°C for 24 h. Table 6 reports the experimental results obtained from these
281 commercial herbal medicines.

282

283 **Table 6.** Mean values of Ir(III), Os(VIII), Pd(II), Pt(II), Rh(III), Ru(III) (ng g⁻¹) for *Eucalyptus*
284 *globulus*, *Harpagophytum procumbens DC* and *Taraxacum officinale weber* sold on the market
285 (Portomaggiore, Italy). Experimental values are calculated as the mean of 5 independent
286 determinations. The confidence level was 95%.

287
288

3.4 Comparison between spectroscopic and voltammetric measurements

289 The results shown in Tables 5 and 6, related to the accuracy (precision and trueness) and the
290 real sample analyses, can be considered as an additional validation of the analytical method, based
291 on voltammetry, herein developed. Such a validation can be deduced from the good agreement

292 between the spectroscopic and voltammetric results. Specifically, as regards precision, trueness and
293 detection limits, no significant difference was observed between results obtained with the two
294 different techniques, and analytical performance was good in all cases. Indeed, spectroscopy was
295 taken as reference technique due to its analytical qualities: a well-established and tested robust
296 procedure, suitable for this purpose [35]. However, voltammetry shows interesting advantages with
297 respect to atomic absorption spectroscopy atomic: almost always, it allows the simultaneous
298 determination of many metals (multi-analytes procedure) in a very easy, rapid and inexpensive way.
299 In particular, the standard addition method, which may be easily applied in the herein proposed
300 voltammetric procedures, makes these procedures very rapid, even for multi-element determinations
301 in complex matrices, without renouncing to good selectivity and good sensitivity. For the sake of
302 correctness, it must be highlighted that both Inductively Coupled Plasma (ICP) and Inductively
303 Coupled Plasma-Mass Spectrometry (ICP-MS) also allow for multi-element analyses, even if ICP-
304 based spectroscopic techniques require time-consuming and expensive sample pre-treatments and
305 enrichment steps, like solvent extraction. Moreover, the instrumentation itself and its maintenance
306 are very expensive (e.g. up to 25-30 times with respect to voltammetry), especially when multi-
307 element spectroscopic determinations are performed.

308

309 **CONCLUSIONS**

310 As highlighted in this work, the method herein developed and validated represents a valid
311 procedure for the quantification of PGMs in samples of natural origin. Particularly important is its
312 effective application to natural derived products such as herbal medicines (*Eucalyptus globulus*,
313 *Harpagophytum procumbens DC* and *Taraxacum officinale weber*) for which there is currently no
314 precise legislation regarding the maximum content of heavy metals, especially for those relating to
315 the group of Platinum. The procedure described here can be a valid starting point for future
316 applications aimed at standardizing and quality control of these products increasingly used today.
317 This is all the more evident from the application of this procedure to certified reference materials,

318 through which it was possible to obtain important information about the potential of the method. The
319 analytical performances show how a simple sample preparation can be used in order to avoid
320 interferences and obtain reproducible and robust results through the application of voltammetric
321 techniques well known and widespread in the various laboratories.

322

323 **ACKNOWLEDGEMENTS**

324 Investigation supported by the University of Bologna (Funds for Selected Research Topics).

325

326 **CONFLICT OF INTEREST**

327 The authors declare that the content of this article has no potential conflict of interest.

328

329 **REFERENCES**

- 330 1 Locatelli, C. Metals. In *Comprehensive Analytical Chemistry*; Picò, Y. Ed.; Elsevier Publ.:
331 Amsterdam, Netherland, 2008; vol. 51, Ch. 16, pp. 571-598.
- 332 2 Ferré-Huguet, N.; Martí-Cid, R.; Schuhmacher, M.; Domingo, J.L. Risk assessment of metals
333 from consuming vegetables, fruits and rice grown on soils irrigated with waters of the Ebro
334 river in Catalonia, Spain. *Biol. Trace Elem. Res.* **2008**, *123*, 66-79.
- 335 3 Woo, C.S.J.; Lau, J.S.H.; El-Nezami, H. Herbal medicines. Toxicity and recent trends in
336 assessing their potential toxic effects. *Adv. Bot. Res.* **2012**, *62*, 365-384.
- 337 4 Yuan, X.; Chapman, R.L.; Wu, Z. Analytical methods for heavy metals in herbal medicines.
338 *Phytochem. Analysis* **2011**, *22*, 189-198.
- 339 5 World Health Organization (WHO). Supplementary guidelines on good manufacturing
340 practices for the manufacture of herbal medicine. Geneva, 2006.
- 341 6 European Medicines Agency (EMA). Guideline on specifications: test procedures and
342 acceptance criteria for herbal substances, herbal preparations and herbal medicinal products /
343 traditional herbal products CPMP/QWP/2820/00 Rev. 1. London EMA, 2006.

- 344 7 Melucci, D.; Casolari, S.; De Laurentiis, F.; Zappi, A.; Locatelli, M.; Locatelli, C. Toxic metals
345 in *Camellia sinensis*: analytical methods, human health risk and regulations. In: *Agricultural*
346 *Research updates*; Gorawala, P.; Mandhatri, S. Editors; Nova Science Publ. Inc.: New York,
347 USA., 2019; Vol. 26, Ch. 3, pp. 99-146.
- 348 8 Locatelli, C.; Melucci, D.; Locatelli, M. Toxic metals in herbal medicines. A review. *Curr.*
349 *Bioact. Compd.* **2014**, *10*(3), 181-188.
- 350 9 Melucci, D.; Torsi, G.; Locatelli, C. Analytical procedure for the simultaneous voltammetric
351 determination of trace metals in food and environmental matrices. Critical comparison with
352 atomic absorption spectroscopic measurements. *Ann. Chim. (Rome)* **2007**, *97*, 141-151.
- 353 10 Zhao, Y.S.; Ma, X.F.; Fan, L.L.; Mao, F.Y.; Tian, H.L.; Xu, R.; Cao, Z.; Zhang, X.H.; Fu,
354 X.Y.; Sui, H. Discrimination of geographical origin of cultivated *Polygala tenuifolia* based on
355 multi-element fingerprinting by inductively coupled plasma mass spectrometry. *Sci. Rep.*
356 **2017**, *7*, 12577.
- 357 11 Wang, H.W.; Liu, Y.Q. Evaluation of trace and toxic element concentrations in *Paris*
358 *polyphylla* from China with empirical and chemometric approaches. *Food Chem.* **2010**, *121*,
359 887-892.
- 360 12 Ma, X.F.; Fan, L.L.; Mao, F.Y.; Zhao, Y.S.; Yan, Y.G.; Tian, H.L.; Xu, R.; Peng, Y.Q.; Sui,
361 H. Discrimination of three *Ephedra* species and their geographical origins based on multi-
362 element fingerprinting by inductively coupled plasma mass spectrometry. *Sci. Rep.* **2018**, *8*,
363 10271.
- 364 13 Esteki, M.; Vander Heyden, Y.; Farajmand, B.; Kolahderazi, Y. Qualitative and quantitative
365 analysis of peanut adulteration in almond powder samples using multi-elemental fingerprinting
366 combined with multivariate data analysis methods. *Food Control* **2017**, *82*, 31-41.
- 367 14 Locatelli, C.; Melucci, D. Critical comparison between voltammetric and spectroscopic
368 techniques for the determination of heavy metals and platinum group metals (PGMs)
369 concentrations in Italian airborne particulate matter. In: *Airborne Particles: Origin, Emissions*

- 370 *and Health Impact*; Kumar, P. Editor, Nova Science Publ. Inc.: New York, USA, 2017; Ch. 9,
371 pp. 135-180.
- 372 15 Locatelli, C.; Melucci, D. Heavy metals and platinum group metals (PGMs) determination in
373 oysters, mussels and clams as bio-monitors of the aquatic system pollution. In: *Oysters and*
374 *Clams: Cultivation, Habitat Threats and Ecological Impact*; Romalde, Jesùs L. Editor, Nova
375 Science Publ. Inc.: New York, USA, 2016; Ch. 9, pp. 145-188.
- 376 16 Locatelli, C.; Melucci, D. Osmium, an emerging anthropic polluting traffic-related metal.
377 Analytical methods for its determination in environmental matrices of interest for human
378 health and involved in the food chain. In: *Osmium: Synthesis, Characterization and*
379 *Applications*; Wise, G. Editor, Nova Science Publ. Inc.: New York, USA, 2016; Ch. 1, pp. 1-
380 42.
- 381 17 Locatelli, C.; Melucci, D. Iridium, the new and recent polluting metal linked to vehicular
382 traffic. Its determination in airborne particulate matter, superficial waters, soils/sediments,
383 vegetables, mussels, clams and algae by voltammetry and spectroscopy: critical comparison.
384 In: *Advances in Environmental Research*; Daniels, J.A. Editor, Nova Science Publ. Inc.: New
385 York, USA, 2015; Ch. 4, pp. 97-128.
- 386 18 Locatelli, C.; Melucci, D. Voltammetric determination of metals as food contaminants- An
387 excellent alternative to spectroscopic measurements: application to meals, vegetable, mussels,
388 clams and fishes. In: *Voltammetry : Theory, Types and Applications*; Saito, Y.; Kikuchi, T.
389 Editors, Nova Science Publ. Inc.: New York, USA, 2014; Ch. 8, pp. 225-248.
- 390 19 Locatelli, C.; Melucci, D. Voltammetric and spectroscopic methods for the ruthenium
391 determination in the environment at ultra-trace concentration level. Critical comparison and
392 application to airborne particulate matter, vegetables, superficial waters, mussels, clams and
393 soils. In: *Ruthenium: Synthesis, Physicochemical Properties and Applications*; Edited by
394 Keeler, GP. Editor, Nova Science Publ. Inc.: New York, USA, 2014;Ch. 6, pp. 207-233.

- 395 20 Locatelli, C. Square wave catalytic adsorptive voltammetric determination of osmium,
396 ruthenium and lead in vegetable environmental biomonitors. *Int. J. Environ. Anal. Chem.* **2014**,
397 *94(3)*, 277-290.
- 398 21 Locatelli, C. Use of peak area instrumental datum as possibility to improve the analytical
399 sensitivity in the sequential voltammetric determination of ultra-trace iridium and lead in
400 vehicle emission particulate matter. *Microchem. J.* **2013**, *110*, 99-106.
- 401 22 Locatelli, C. Simultaneous determination of osmium, ruthenium, copper and lead by
402 electrocatalytic voltammetry. Application to superficial waters. *Microchem. J.* **2012**, *102*, 54-
403 60.
- 404 23 Locatelli, C. Ultratrace osmium, ruthenium and lead in airborne particulate matter: peak area
405 as instrumental datum to improve their simultaneous voltammetric determination.
406 *Electroanalysis* **2012**, *24*, 2273-2282.
- 407 24 Locatelli, C. Sequential voltammetric determination of ultratrace osmium, ruthenium and
408 iridium Application to superficial water. *Electroanalysis* **2011**, *23*, 1329-1336.
- 409 25 Locatelli, C. Voltammetric peak area as instrumental datum. A possibility to improve the
410 determination at ultratrace level concentration of platinum group metals (PGMs) and lead.
411 Application to particulate matter. *Electroanalysis* **2007**, *19(4)*, 445-452.
- 412 26 Melucci, D.; Locatelli, C. Platinum(II), palladium(II), rhodium (III) and lead(II) voltammetric
413 determination in sites differently influenced by vehicle traffic. *Ann. Chim. (Rome)* **2007**, *97*,
414 373-384.
- 415 27 Locatelli, C. Voltammetric analysis of trace levels of platinum group metals – principles and
416 applications. *Electroanalysis* **2007**, *19(21)*, 2167-2175.
- 417 28 Locatelli, C. Simultaneous square wave stripping voltammetric determination of platinum
418 group metals (PGMs) and lead at trace and ultratrace concentration level. Application to
419 surface water. *Anal. Chim. Acta* **2006**, *557(1-2)*, 70-77.

- 420 29 Locatelli, C. Platinum, rhodium, palladium and lead: elements linked to vehicle emissions.
421 Their simultaneous voltammetric determination in superficial water. *Electroanalysis* **2005**,
422 *17*(2), 140-147.
- 423 30 Locatelli, C.; Melucci, D.; Torsi, G. Determination of platinum-group metals and lead in
424 vegetable environmental bio-monitors by voltammetric and spectroscopic techniques: critical
425 comparison. *Anal. Bioanal. Chem.* **2005**, *382*(7), 1567-1573.
- 426 31 Melucci, D.; Locatelli, M.; De Laurentiis, F.; Zengin, G.; Locatelli, C. Herbal medicines:
427 Application of a sequential voltammetric procedure to the determination of mercury, copper,
428 lead, cadmium and zinc at trace level. *Lett. Drug Des. Discov.* **2018**, *15*, 270-280.
- 429 32 Welz, B.; Sperling, M. *Atomic Absorption Spectrometry*, 3rd ed.; Wiley VCH: Weinheim
430 (Germany), 1999.
- 431 33 International Union of Pure and Applied Chemistry, Analytical Chemistry Division.
432 Nomenclature, symbols, units and their usage in spectrochemical analysis. II. Data
433 interpretation. *Spectrochim. Acta - Part B* **1978**, *33*, 241-245.
- 434 34 Miller, J.C.; Miller, J.N. *Statistics and Chemometrics for Analytical Chemistry*, 6th ed.;
435 Pearson Education Ltd. Publ., Ashford Colour Press Ltd: Gosport, U.K., 2010.
- 436 35 Ferreira, S.L.C.; Korn, M.G.A.; Ferreira, H.S.; da Silva, E.G.P.; Araujo, R.G.O.; Souza, A.S.;
437 Macedo, S.M.; Lima, D.C.; de Jesus, R.M.; Amorim, F.A.C.; Bousque-Sendra, J.M.
438 Application of multivariate techniques in optimization of spectroanalytical methods. *Appl.*
439 *Spectrosc. Rev.* **2007**, *42*, 475-491.