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NEW POLLUTING METALS. QUANTIFICATION IN HERBAL MEDICINES BY VOLTAMMETRIC AND SPECTROSCOPIC ANALYTICAL METHODS

--Manuscript Draft--

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Dear Editor

Enclosed please find the revised version of the manuscript "**NEW POLLUTING METALS. QUANTIFICATION IN HERBAL MEDICINES BY VOLTAMMETRIC AND SPECTROSCOPIC ANALYTICAL METHODS**", submitted to the **Journal of Pharmaceutical and Biomedical Analysis**, as a Full Article for the **Special Issue devoted to the XVII IHSS (VSI: Health Protection)**.

The present version was checked accordingly to the Editor and Reviewers comments. Authors thank Editor and Reviewers for their positive evaluations and for the suggestions that were all accepted and reported in the revised version. Below are reported our point-by-point response to the Reviewers comments.

The reported manuscript fit with journal aims because it included sampling, sample preparation, herbal medicine analyses and applications to real samples. The manuscript has been read and approved by all authors, who declare no conflict of interest. This research has not been disclosed or published and is not under consideration for publication elsewhere.

Sincerely,
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Analytical and Bioanalytical Chemistry
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Editor

Please follow the reference rules carefully: reference style is as follows: Please keep in your mind the reference number should not exceed 35 for a full-length (research) manuscript and 25 references for short communication. There is no limit for Review articles. Text: Indicate references by number(s) in square brackets in line with the text. The actual authors can be referred to, but the reference number(s) must always be given.
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Dear Editor, firstly thanks a lot for your evaluation. Authors have checked and revised all details following Editor/Journal recommendations.

Reviewer #1

Reviewer #2

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The Authors thank the Reviewer for the positive evaluation of the work.

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The Authors thank the Reviewer for this comment. As correctly suggested the sentence was revised in: "Similarly, Zn(II) may interfere during Pt(II) quantification (Figure 1b), showing a voltammetric …"

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Highlights

- ✓ New application for the determination of ultra-trace Platinum Group Metals (PGMs)
- ✓ Herbal medicines analyses
- ✓ Sample treatment for the voltammetric technique application to real samples
- ✓ Potential application to quality control and safety studies

Abstract

A new application of voltammetric techniques in stripping mode to the quantitative determination of metals belonging to the platinum group (PGM) in herbal medicines, at the ultra-trace level, is reported. Pd (II), Pt (II) and Rh (III) are determined by means of square-wave adsorption voltammetry (SWAdSV); Os (VIII) and Ru (III) are determined through square-wave voltammetry in catalytic mode (SWCV); Ir (III) is determined through the application of square-wave catalytic voltammetry in adsorption mode (SWAdCSV). In all the applied methods, the voltammetric cell has the conventional setting with three-electrodes, which sees the presence of a suspended mercury-drop electrode (HMDE) or a glassy carbon electrode (GCE) as working electrodes for the determination of Ir (III). The auxiliary electrode was a platinum electrode, and an $Ag|AgCl|KCl_{satd.}$ electrode was employed as reference electrode.

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NEW POLLUTING METALS.

QUANTIFICATION IN HERBAL MEDICINES BY VOLTAMMETRIC AND SPECTROSCOPIC ANALYTICAL METHODS

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Keywords: PGMs, Voltammetry, Spectroscopy, Herbal Medicines.

1. INTRODUCTION

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

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

Herbal medicines could appear as not dangerous to health, being derived from natural products. Unfortunately, at now the scientific evidences are not enough to support this general belief. While synthetic products can be purified and stabilized in their formulations, with a defined dosage to obtain the therapeutic effect and minimizing the adverse effects, herbal medicines are difficult to standardize due to raw extracts containing different active ingredients (phytocomplex). This is mainly because different pharmacologically active ingredients are present in the phytocomplex, often similar to synthetic compounds but potentially showing a greater number of adverse effects [1-3].

In the specific case of PGMs, the major problem is linked to their long persistence time in the atmosphere which can lead to a high level of bioaccumulation in the environmental matrices (plants, terrestrial and aquatic organisms). This appears to be one of the possible main causes of serious damage to food safety, as well as one of the preferential routes, in addition to inhalation, of exposure of human beings (as final consumers of the supply chain). In fact, in the human organism there are metabolic processes capable of converting these xenobiotics into non-toxic forms which are subsequently excreted. As long as the absorption rate (even if integrated through dietary sources and herbal medicine) is lower than the conversion and excretion rate, the phenomenon of bioaccumulation will not be observed and the risk of toxicity phenomena will be reduced. The opposite case is found when the speed of absorption is greater than the speed of its excretion and detoxification. In this case, the phenomenon of bioaccumulation is generally observed with consequent exceeding of the "critical" threshold, and the compounds will begin to bind in the sites where they will interfere with normal metabolic functioning. The major risk currently lies in the fact that PGMs, although still considered as micronutrients (and for this reason not fully investigated in terms of toxicological profiles), at high concentrations may express potential toxicity (in all their different bioavailable forms) [4]. For this reason, monitoring and quality control processes in the food supply chain is even more imperative, especially in the sector that includes herbal medicines and food supplements, which is poorly monitored and regulated. Another element not to be overlooked is that, to date, there is no real regulation for the production of herbal medicines, combined with the fact that they can be purchased without a prescription. In light of these regulatory deficiencies, international organizations like WHO (World Health Organization) and FDA (US Food and Drug Administration) simply recommend to check for the quantity of toxic heavy metals in medicinal plants, starting from raw materials. Only for some of these metals the upper-threshold admissible limits are defined as mg L^{-1}/µg g^{-1} for mercury (0.1), lead (3.0), cadmium (1) [5-6]. For all other metals, a real decision has not been evaluated and/or taken, yet.

What so far discussed should fully fall within the pharmacovigilance, i.e. the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem. Unfortunately, pharmacovigilance has been introduced and designed mainly for synthetic drugs. In the field of herbal medicines, where the market develops (often in an uncontrolled way) especially through products that do not require a prescription (even with mail order and internet sales) and considering the possible problems related to public health deriving from uncontrolled products and/or regulated with regard to metals and upper-threshold admissible limits, it is increasingly evident that research in this area plays a fundamental role in safeguarding human health [7].

In the analysis of metals, the most widely used instrumental technique is certainly the electrothermal atomic absorption spectroscopy (ET-AAS) [8-10], especially in the configuration that envisages the graphite furnace (GF-AAS) as atomization device. In the literature there are some works [11, 12] in which the herbal medicine fingerprint is obtained using this technique, which can then be processed using chemometric approaches to investigate their origin [13], or the possible investigation of frauds [14].

In recent years, a line of research has been expanded in our laboratories that includes investigating new approaches to quantitatively determine PGMs in environmental and food samples [15-28] through the application of spectroscopic techniques, and only recently to quantify toxic metals in matrices characterizing this sample categories [9, 29].

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

2. EXPERIMENTAL

2.1 Instrumentation

The acquisition of the voltammograms was performed by a Multipolarograph AMEL Mod. 433 (Milan, Italy). The measuring cells (three-electrode set-up) is structured in the case of iridium with a working electrode consisting in a glassy-carbon electrode (GCE, AMEL, Milan; 7.065-mm^2surface area). For all the other PGMs herein considered, a stationary HMDE was used. In all cases, Ag|AgCl|$KCl_{satd.}$ was the reference electrode and the auxiliary electrode was a platinum-wire electrode. Several rinses of the Teflon voltammetric cell were performed before carrying out measurements, in order to prevent contamination. This procedure was performed as follow: *i)* a first rinsing with supra-pure HNO_3 $69\%_{w/w}$, diluted 1:1 with water obtained by a Milli-Q deionizing system (Millipore, Darmstadt, Germany); *ii)* the cell was accurately rinsed by Milli-Q water.

Disposable plastic tips were used for the standard additions. The solutions were previously thermostated at 20.0±0.5°C and treated by pure N_2 for 5 min to remove oxygen. During the measurements, solutions were kept under nitrogen. The magnetic stirring (with Teflon-coated magnetic stirring bar) was constantly maintained during the purge step. For the analyses here presented, the supporting electrolytes and the voltammetric parameters are reported in **Table 1**.

An Atomic Absorption Spectrometer by Perkin-Elmer (Mod. A-Analyst 100 with deuterium background-corrector, Autosampler AS-72 and graphite furnace model HGA 800), was used for the acquisition of ET-AAS measurements. The light sources were Lumina hollow-cathode lamps for single-element measurements (Perkin-Elmer, USA), while the steps of ashing and atomization (different from element to element) were set up as reported in the literature [30]. The instrument settings used were those suggested by the Manufacturer [31] with minor changes.

2.2 Reagents, Reference Solutions, and real samples

Chemicals at suprapure-grade (Merck, Darmstadt, Germany) were used in this work, while a Milli-Q system was use to obtain demineralized water. Reference standard solutions were obtained starting from acidic standard solutions: Ir(III), Pd(II), Pt(II), Rh(III), Ru(III) (1000 mg L^{-1}, Merck, Darmstadt, Germany). Osmium tetroxide solution in water (4%$_{w/w}$, Sigma-Aldrich, Darmstadt, Germany) was used as starting standard solution. In order to optimize the instrumental parameters, two reference materials were used as standard: NIST-SRM 1570a (Spinach Leaves) and NIST-SRM 1573a (Tomato Leaves) (National Institute of Standards and Technology, Gaithersburg, MD, USA). The real samples of *Eucalyptus globulus*, *Harpagophytum procumbens DC* and *Taraxacun officinale weber* analyzed by the herein reported procedure were sold on the local market (Portomaggiore, Italy).

2.3 Sample Preparation

The standard reference materials (NIST-SRM 1570a and NIST-SRM 1573a) and the herbal medicines (*Eucalyptus globulus*, *Harpagophytum procumbens DC* and *Taraxacum officinale Weber*) were solubilized by an acidic attack using a mixture of HCl, HNO_3 and H_2SO_4 (3mL:7mL:5mL, respectively).

The vegetables were accurately weighed (order of magnitude: 1 g), and put in a 25-mL Pyrex-tube for digestion, and a solution containing 4 mL HNO_3 69%$_{w/w}$, 4 mL HCl 37%$_{w/w}$ and 5 mL H_2SO_4 96% $_{w/w}$ was added. Then a Vigreux column condenser was connected to the tube , and the tube was inserted into a cold block digester (appositely homemade); finally, the temperature was progressively raised up to 150°C and maintained at this final temperature for 2 h to achieve the mineralization. After this step, the content was dried, cooled, and dissolved in Milli-Q water (25 mL) or in 25 mL of the supporting electrolyte.

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

For better readability of the text, from this point on the molar concentration (mol ~~l~~L^{-1}) is indicated with M.

The voltammetric procedure for Pd(II), Pt(II) and Rh(III) comprises two steps: *i)* 10-mL sample aliquot of 0.15 M HCl + $1.9\cdot10^{-4}$ M dimethylglyoxime (DMG) aqueous reference solution or solutions obtained in the mineralization step were transferred into the voltammetric cell; de-aeration was achieved by bubbling water-saturated pure N_2 for 5 min. The determination of Pd(II) was carried out by SWAdSV; *ii)* 0.5 mL of $1.37\cdot10^{-2}$ M formaldehyde + $2.63\cdot10^{-2}$ M hydrazine (formazone complex) in 0.15 M HCl solution (final concentration: formaldehyde = 0.65 mM and hydrazine = 1.25 mM) were transferred in the cell. Pt(II) and Rh(III) were determined by SWAdSV.

2.5 2.5 Possible, even if unlikely, interference from Pb(II) and Zn(II) in quantifying Pd(II) and Pt(II) by voltammetry

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

Similarly, Zn(II) may interfere during Pt(II) quantification~~,~~ **(Figure 1b)**, showing a voltammetric peak at –0.877 V *vs.* Ag|AgCl|KCl$_{satd.}$. Again, the Zn(II) electrode process shows a low reversibility degree in the employed supporting electrolyte, and the interference can observed only in the case of very high concentrations ratios ($c_{Zn(II)}:c_{Pt(II)} > 750$ μg g^{-1}).

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

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

i) 10-mL sample aliquots of 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$ aqueous reference solution, or of solutions obtained in the mineralisation step containing 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$, were transferred into the cell; de-aeration was kept for 5 min by bubbling water-saturated pure N_2. The determination of Os(VIII) and Ru(III) was performed by SWCV with HMDE electrode

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

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

Cu(II) (-0.169±0.015 V *vs.* Ag|AgCl|$KCl_{satd.}$) and Pb(II) (-0.327±0.010 V *vs.* Ag|AgCl|$KCl_{satd.}$) in 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$ supporting electrolyte could be strongly interfering species in the determination of Os(VIII) (-0.243 ±0.010 V *vs.* Ag|AgCl|$KCl_{satd.}$) and Ru(III) (-0.023 ±0.015 V *vs.* Ag|AgCl|$KCl_{satd.}$). This phenomenon, reported in **Figure 2a**, is decidedly important also related to the fact that Cu(II) and Pb(II), in general, are ubiquitary in all real matrices, especially the environmental ones, also at low levels of concentration. This drawback can be avoided using the well-known ability of Ethylene Diamine Tetraacetic Acid (EDTA) to shift peaks toward cathodic potentials if added to the voltammetric cell. In this work, Cu(II) and Pb(II) interfering peaks were shifted by adding EDTA di-sodium salt, specifically: 200 µL EDTA-Na_2 $9.6 \cdot 10^{-3}$ M were added to a 10-mL sample aliquot, obtaining $1.88 \cdot 10^{-4}$ M as final EDTA-Na_2 concentration. Indeed, the EDTA-Na_2 presence results in a shift of potential values in the cathodic direction, either for Cu(II) and Pb(II) or Os(VIII) and Ru(III) [Cu(II): -0.301±0.015; Pb(II): -

0.751±0.010; Os(VIII): -0.096±0.015; Ru(III): -0.529±0.015 V *vs.* Ag│AgCl│KCl$_{satd.}$]. Under these conditions, the final position of the four peaks relevant to these elements makes it possible to achieve complete resolution (see **Figure 2b**), and consequently the quantitative determination becomes possible.

As for Ir(III), it seems that the presence of EDTA-Na$_2$ slightly changes the position [Ir(III) peak potential with EDTA-Na$_2$ $1.88 \cdot 10^{-4}$ M: +0.623±0.015 V *vs.* Ag│AgCl│KCl$_{satd.}$; Ir(III) peak potential without EDTA-Na$_2$: +0.609±0.010 V *vs.* Ag│AgCl│KCl$_{satd.}$], keeping the peak height practically unchanged, and without interference peaks. Following these experimental observations, several elements can be smoothly determined by the procedure suggested here.

3. RESULTS AND DISCUSSION

3.1 Aqueous Reference Solutions

The calculation of limits of detection (LOD) and limits of quantification (LOQ) (**Table 2**) was performed according to IUPAC by the "3 σ approach", were σ is the observed standard deviation. LODs were calculated in the aqueous reference solution and in the digestates obtained from standard reference materials. LODs 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 the slope of the analytical calibration function. Similarly, the LOQs were evaluated as $(K\ s_{y/x})/b$ (K=6).

When voltammetry was used, LODs were obtained directly in the real matrices (**Table 2**); in fact, the standard addition method was applied to create the analytical calibration model. For each analyte, linearity was explored through regression models of voltammetric signal *vs.* metal concentration in aqueous reference solution with the instrumental details reported in ~~Tables~~Table **1**. The linearity of the model was demonstrated in the range from LOQs up to 10.0 µg L^{-1}. Determination coefficients R^2 were always higher than 0.9989.

3.2 Quality control and quality assessment

Standard reference materials of Spinach Leaves and Tomato Leaves were analysed by means of voltammetric and spectroscopic method for validating the whole method through the evaluation of its accuracy (**Table 3**). Furthermore, the repeatability was evaluated as relative standard deviation (s_r%) [32-33], from 5 independent measurements, and always resulted better than 6%. Trueness was evaluated through the relative error (*e*%), and resulted to be on the order of 5-7%. These experimental values observed for the method correspond to satisfactory accuracy.

3.3 Practical applications

Since the quantification of PGMs in reference materials gave good accuracy, the methods were applied to real samples: herbal medicines sold on the local market, and based on *Eucalyptus globulus*, *Harpagophytum procumbens DC* and *Taraxacun officinale weber*. The section "Sample Preparation" describes how samples were treated, after a preliminary step of powdering, by lyophilisation and dried at 80°C for 24 h. **Table 4** reports the experimental results obtained from these commercial herbal medicines in terms of mean concentration values obtained by applying the herein proposed and validated procedure (both voltammetry and spectroscopy).

As can be seen from the results, the two applied techniques provide absolutely comparable quantitative results and represent an excellent starting point for monitoring and quality control on raw materials used in the herbal medicine sector. It should also be noted that the PGMs detected in the herbal medicines considered herein show high levels for Pt(II) and Pd(II) (about 20-30 ~~(~~μg g^{-1}), while Ir(III) is shown to be the least abundant (about 2-5 μg g^{-1}~~) .~~). Intermediate values are instead observed for Rh(III), Os(VIII), and Ru(III) (about 7-15 μg g^{-1}). These values, considering the anthropogenic activities, account for the trend of transition from platinum, palladium and rhodium-based catalysts to the increasing use of iridium, ruthenium and, above all, osmium [1].

In addition, when compared with the average values estimated at the lithosphere level (0.015 μg g^{-1} for palladium, 0.0001 μg g^{-1} for rhodium, 0.0001 μg g^{-1} for ruthenium, 0.005 μg g^{-1} for osmium, and 0.001 μg g^{-1} for iridium [34]) it is well understood how their bioaccumulation can lead to serious problems regarding public health and the importance of evaluating the inclusion of upper -threshold admissible limits, just as was done for mercury and other metals. All these elements must in fact be correlated, evaluated, and regulated not only considering these metals as micronutrients, but also evaluating their potential harmfulness when they are assumed in large quantities not only from the surrounding environment, but also as a result of the wide use that nowadays makes supplements and herbal medicines that are easily accessible to anyone and without prescription.

3.4 Comparison between spectroscopic and voltammetric measurements

The results shown in **Tables 3** and **4**, related to the accuracy (precision and trueness) and the real sample analyses, can be considered as an additional validation of the analytical method, based on voltammetry, herein developed. Such a validation can be deduced from the good agreement between the spectroscopic and voltammetric results. Specifically, as regards precision, trueness and detection limits, no significant difference was observed between results obtained with the two different techniques, and analytical performance was good in all cases. Indeed, spectroscopy was taken as reference technique due to its analytical qualities: a well-established and tested robust procedure, suitable for this purpose [35]. However, voltammetry shows interesting advantages with respect to atomic absorption spectroscopy atomic: almost always, it allows the simultaneous determination of many metals (multi-analytes procedure) in a very easy, rapid and inexpensive way. In particular, the standard addition method, which may be easily applied in the herein proposed voltammetric procedures, makes these procedures very rapid, even for multi-element determinations in complex matrices, without renouncing to good selectivity and good sensitivity. For the sake of correctness, it must be highlighted that both Inductively Coupled Plasma (ICP) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) also allow for multi-element analyses, even if ICP-based spectroscopic techniques require time-consuming and expensive sample pre-treatments and enrichment steps, like solvent extraction. Moreover, the instrumentation itself and its maintenance are very expensive (e.g. up to 25-30 times with respect to voltammetry), especially when multi-element spectroscopic determinations are performed.

CONCLUSIONS

As highlighted in this work, the method herein developed and validated represents a valid procedure for the quantification of PGMs in samples of natural origin. Particularly important is its effective application to natural derived products such as herbal medicines (*Eucalyptus globulus*, *Harpagophytum procumbens DC* and *Taraxacun officinale weber*) for which there is currently no precise legislation regarding the maximum content of heavy metals, especially for those relating to the group of Platinum. The procedure described here can be a valid starting point for future applications aimed at standardizing and quality control of these products increasingly used today. This is all the more evident from the application of this procedure to certified reference materials, through which it was possible to obtain important information about the potential of the method. The analytical performances show how a simple sample preparation can be used in order to avoid interferences and obtain reproducible and robust results through the application of voltammetric techniques well known and widespread in the various laboratories.

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CONFLICT OF INTEREST

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

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FIGURE CAPTIONS:

Figure 1. SWAdSV. a) determining Pd(II) in the presence of Pb(II) in *Taraxacun officinale weber*. Supporting electrolyte: 0.15 M HCl + $1.9 \cdot 10^{-4}$ M DMG. Pd(II) corresponds to Peak 1; Pb(II) corresponds to peak 2 ; b) determination of Pt(II) and Rh(III) in the presence of Zn(II) in *Taraxacun officinale weber*. Supporting electrolyte: 0.15 M HCl + $1.9 \cdot 10^{-4}$ M DMG + 0.65 mM formaldehyde + 1.25 mM hydrazine (formazone complex in 0.15 M HCl). Peak 1 corresponds to Zn(II), peak 2 corresponds to Pt(II), peak 3 corresponds to Rh(III). Experimental conditions: see Table 1

Figure 2. SWCV. Os(VIII) and Ru(III) in presence of Cu(II) and Pb(II) in *Taraxacun officinale weber*. Supporting electrolyte: 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$ in absence (a) and presence (b) of EDTA-Na_2. Peak 1 corresponds to Os(VIII), peak 2 corresponds to Cu(II), peak 3 corresponds to Ru(III), peak 4 corresponds to Pb(II). Experimental conditions: see Table 2.

The other authors declare that does not exist any economic interest or any conflict of interest.

Figure 1

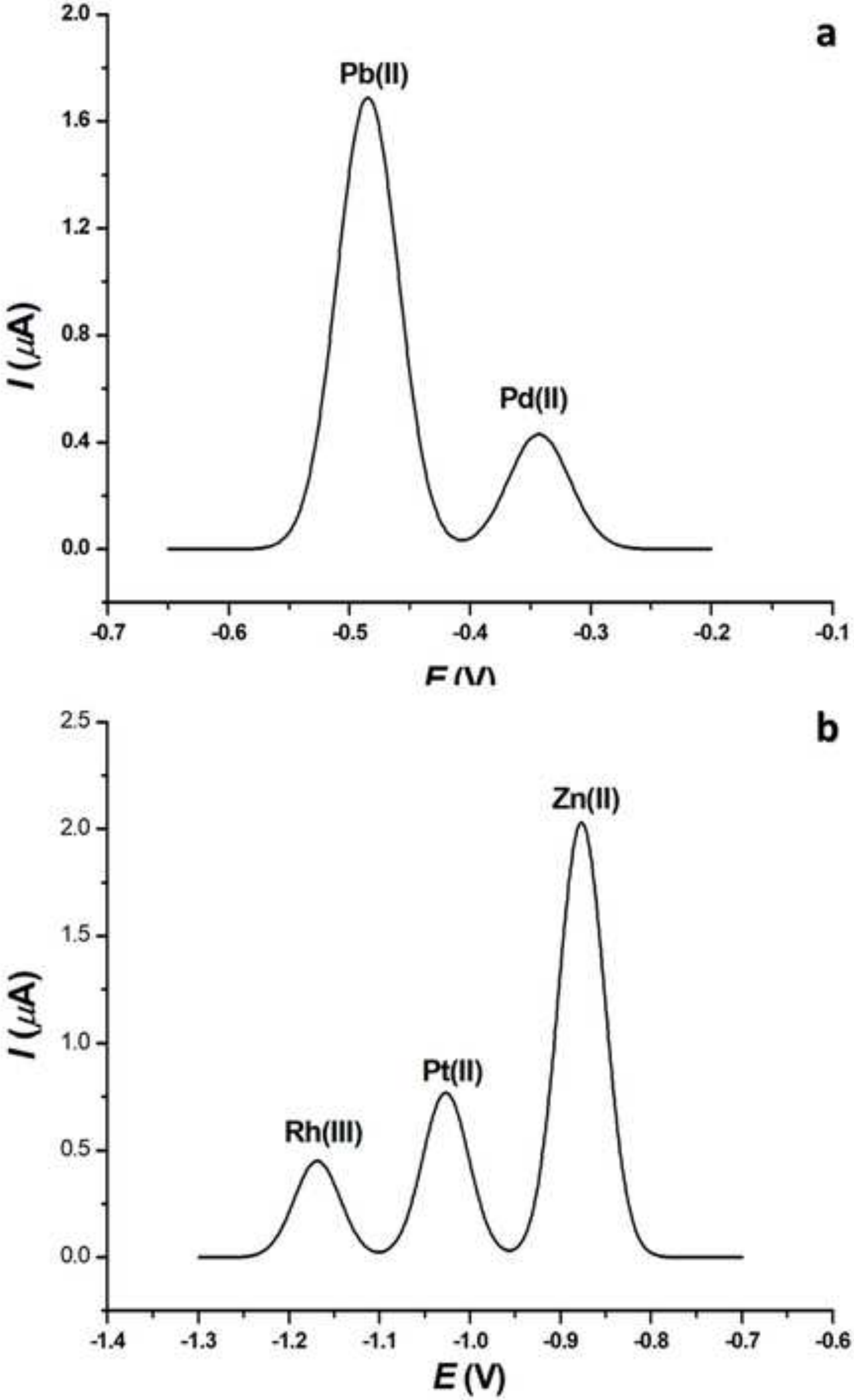
a
Pb(II)
Pd(II)
I (μA)
2.0
1.6
1.2
0.8
0.4
0.0
-0.7
-0.6
-0.5
-0.4
-0.3
-0.2
-0.1
E (V)
b
Zn(II)
Pt(II)
Rh(III)
I (μA)
2.5
2.0
1.5
1.0
0.5
0.0
-1.4
-1.3
-1.2
-1.1
-1.0
-0.9
-0.8
-0.7
-0.6
E (V)

Figure 2

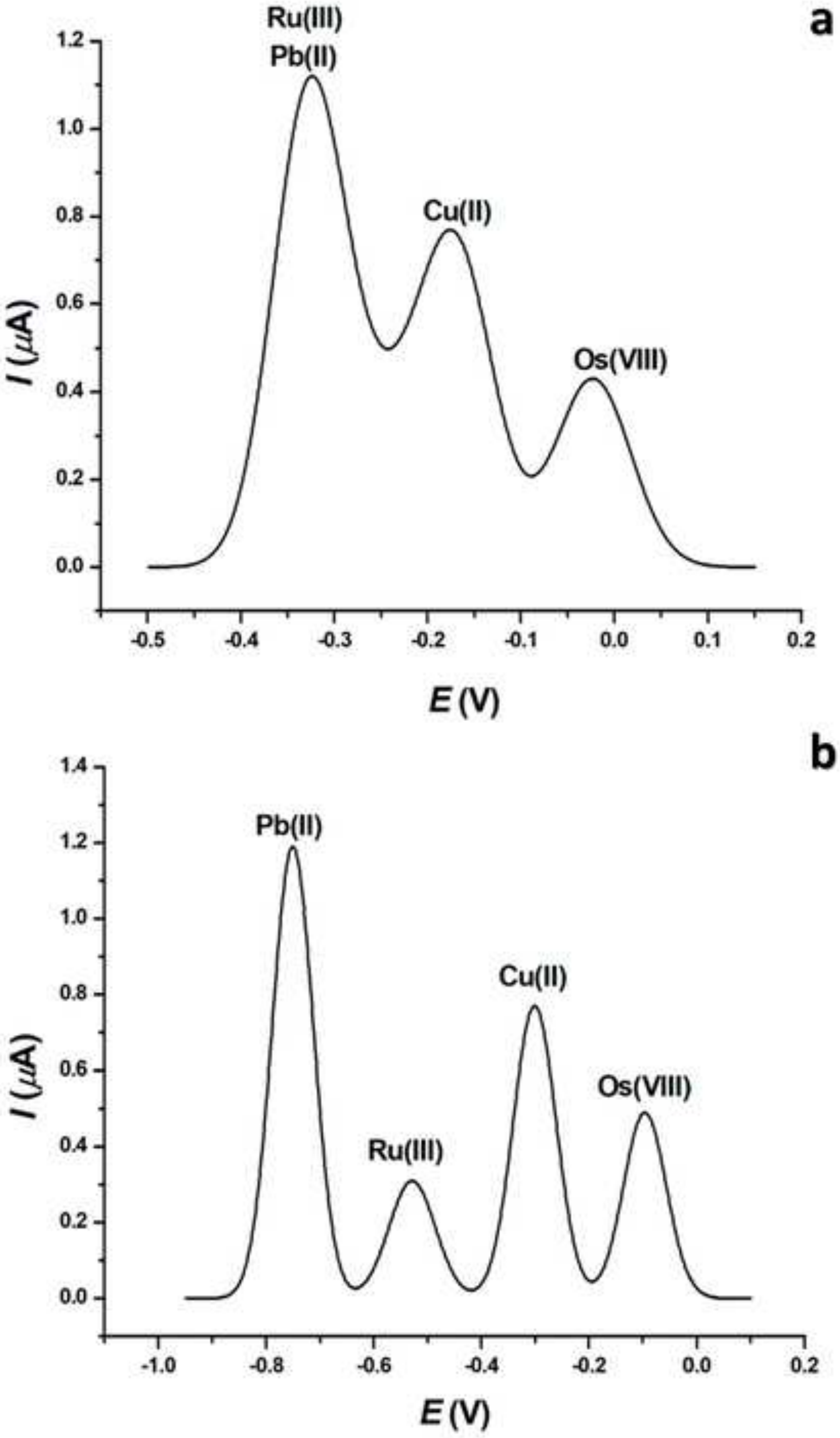
a
Ru(III)
Pb(II)
Cu(II)
Os(VIII)
I (μA)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
E (V)
b
Pb(II)
Ru(III)
Cu(II)
Os(VIII)
I (μA)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
E (V)

Table 1. Settings of instrumentation. Electrolytic solutions: *i)* for Pb(II) by SWASV, Pd(II), Pt(II) and Rh(III) by SWAdSV: 0.15 M HCl + $1.9\cdot10^{-4}$ M dimethylglyoxime [Pd(II)] and 0.15 M HCl + $1.9\cdot10^{-4}$ M dimethylglyoxime + 0.65 mM formaldehyde + 1.25 mM hydrazine (formazone complex) in 0.15 M HCl [Pt(II) and Rh(III)] *ii)* for Ir(III) by SWAdCSV: 0.5 M acetate buffer pH 4.9 + $7.3\cdot10^{-2}$ M $NaBrO_3$ + $4.9\cdot10^{-5}$ M cetyltrimethylammonium bromide + 0.25 M KCl *iii)* for Os(VIII) by SWCV and Ru(III): 0.5 M acetate buffer pH 4.9 + $7.3\cdot10^{-2}$ M $NaBrO_3$.

	Pd(II) (SWAdSV)	Pt(II)-Rh(III) (SWAdSV)	Os(VIII)-Ru(III) (SWCV)	Ir(III) (SWAdCSV)
E_i	-0.100	-0.650	+0.150	+0.800
E_a	-0.100	-0.650	+0.150	-
E_d	-	-	-	+0.800
E_f	-0.700	-1.400	-0.550	+0.400
t_a	270	300	360	-
t_d	-	-	-	420
t_r	10	10	10	10
dE/dt	100	100	100	100
ΔE	50	50	50	50
τ	0.010	0.010	0.010	0.010
ν	0.100	0.100	0.100	0.100
η	10	10	10	10
r	600	600	600	600

E_i: initial potential (V/ Ag|AgCl|$KCl_{satd.}$); E_a: adsorption potential (V/ Ag|AgCl|$KCl_{satd.}$); E_d: deposition potential (V/ Ag|AgCl|$KCl_{satd.}$); E_f: final potential (V/ Ag|AgCl|$KCl_{satd.}$); t_a: electroadsorption time (s); t_d: electrodeposition time (s); t_r: delay time before the potential sweep (s); dE/dt: potential scan rate (mV/s); ΔE: step amplitude (mV); τ: sampling time (s); ν: wave period (s); η: wave increment (mV); r: stirring rate (r.p.m.).

Table 2. LODs and LOQs of Ir(III), Os(VIII), Pd(II), Pt(II), Rh(III), Ru(III), in aqueous reference solutions and in solutions from reference materials. Experimental values are the mean of five independent determinations. Confidence level: 95 %.

Medium	**Limits of detection [c]**					
	Pt(II)	Pd(II)	Rh(III)	Os(VIII)	Ru(III)	Ir(III)
Aqueous Reference Solutions	0.21[a]	0.25[a]	0.23[a]	0.31[b]	0.27[b]	0.35[b]
Spinach Leaves NIST-SRM 1570a	7.7	8.5	6.9	9.6	12	12
Tomato Leaves NIST-SRM 1573a	8.1	8.9	8.7	11	12	13
	Limits of quantification [c]					
Aqueous Reference Solutions	0.42[a]	0.50[a]	0.46[a]	0.62[b]	0.54[b]	0.70[b]
Spinach Leaves NIST-SRM 1570a	15	17	14	19	23	25
Tomato Leaves NIST-SRM 1573a	16	18	17	21	24	25

[a] 0.15 M HCl + $1.9 \cdot 10^{-4}$ M DMG for Pd(II) determination with the addition of 0.65 mM formaldehyde + 1.25 mM hydrazine (formazone complex) in 0.15 M HCl for Pt(II) and Rh(III) determination. [b] 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$ [Os(VIII) and Ru(III)], and 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$ + $4.9 \cdot 10^{-5}$ M CTAB + 0.25 M KCl [Ir(III)]. [c] The concentrations are expressed in μg L^{-1} in the aqueous reference solutions and calculated in μg L^{-1} and expressed in ng g^{-1} in the standard reference materials.

Table 3. Accuracy of the analytical procedure. Experimental values are calculated as the mean of 5 independent determinations. The confidence level was 95%. The spiked-sample concentration was always 79.5 ng g^{-1} (the addition to the Standard Reference Materials took place when digestion started).

	Metal	*Voltammetry*			*Spectroscopy*		
		Determined Concentration	e (%)	s_r (%)	Determined Concentration	e (%)	s_r (%)
Spinach Leaves NIST-SRM 1570a	Pt(II)	83.9±4.9	+5.5	5.1	84.5±5.3	+6.3	5.6
	Pd(II)	84.3±5.1	+6.0	5.3	74.7±5.2	-6.0	5.9
	Rh(III)	75.0±5.0	-5.7	5.0	74.5±5.5	-6.3	5.7
	Os(VIII)	74.7±5.4	-6.0	5.5	84.5±5.4	+6.3	5.7
	Ru(III)	74.8±5.1	-5.9	5.9	74.6±5.6	-6.2	6.1
	Ir(III)	84.1±5.2	+5.8	5.8	84.7±5.8	+6.5	6.0
Tomato Leaves NIST-SRM 1573a	Pt(II)	84.0±5.3	+5.7	5.8	74.5±5.5	-6.3	5.8
	Pd(II)	74.9±5.0	-5.8	5.0	84.4±5.4	+6.2	6.0
	Rh(III)	84.2±5.2	+5.9	5.5	84.6±5.7	+6.4	5.9
	Os(VIII)	84.2±5.3	+5.9	5.5	74.4±5.6	-6.4	5.9
	Ru(III)	74.5±5.7	-6.3	5.7	84.7±5.9	+6.5	5.6
	Ir(III)	74.6±5.5	-6.2	5.4	74.8±5.7	-5.9	5.8

Table 4. Mean concentration values (μg g^{-1}) of Ir (III), Os (VIII), Pd (II), Pt (II), Rh (III), Ru (III) for *Eucalyptus globulus*, *Harpagophytum procumbens DC* and *Taraxacun officinale weber* sold on the market (Portomaggiore, Italy). Experimental values are calculated as the mean of 5 independent determinations. The confidence level was 95%.

		Pt(II)	**Pd(II)**	**Rh(III)**	**Os(VIII)**	**Ru(III)**	**Ir(III)**
Voltammetry	*Taraxacum officinale weber*	27.3±1.2	25.1±1.7	13.6±1.0	9.6±0.7	10.3±0.8	4.9±0.6
	Eucalyptus globulus	31.5±1.5	29.9±2.1	15.0±0.8	10.3±0.8	11.5±0.7	5.5±0.7
	Harpagophytum procumbens DC	19.7±1.7	16.5±1.5	10.7±1.1	7.7±0.8	8.0±0.7	2.7±0.5
Spectroscopy	*Taraxacum officinale weber*	26.7±1.3	26.0±1.4	14.1±0.9	10.1±0.6	10.8 ±0.7	4.7±0.5
	Eucalyptus globulus	32.6±1.5	31.8±2.0	15.5±0.7	11.0±0.9	12.1±0.8	5.1±0.6
	Harpagophytum procumbens DC	20.3±1.4	15.3±1.4	11.3±0.9	7.2±0.7	8.5±0.6	2.3±0.6

Author Statement

The other authors declare that does not exist any economic interest or any conflict of interest.

NEW POLLUTING METALS.
QUANTIFICATION IN HERBAL MEDICINES BY VOLTAMMETRIC AND SPECTROSCOPIC ANALYTICAL METHODS

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Abstract

A new application of voltammetric techniques in stripping mode to the quantitative determination of metals belonging to the platinum group (PGM) in herbal medicines, at the ultra-trace level, is reported. Pd (II), Pt (II) and Rh (III) are determined by means of square-wave adsorption voltammetry (SWAdSV); Os (VIII) and Ru (III) are determined through square-wave voltammetry in catalytic mode (SWCV); Ir (III) is determined through the application of square-wave catalytic voltammetry in adsorption mode (SWAdCSV). In all the applied methods, the voltammetric cell has the conventional setting with three-electrodes, which sees the presence of a suspended mercury-drop electrode (HMDE) or a glassy carbon electrode (GCE) as working electrodes for the determination of Ir (III). The auxiliary electrode was a platinum electrode, and an Ag|AgCl|$KCl_{satd.}$ electrode was employed as reference electrode.

Validation of the analytical procedure here proposed has been achieved using reference standards: NIST-SRM 1570a (Spinach Leaves) and NIST-SRM 1573a (Tomato Leaves), both added with pure metal standards, obtaining satisfying precision values, better than the limits set for the validation of quantitative methods. Following the verification of the validity of the here presented procedure, commercially available herbal medicines, based on *Eucalyptus globulus, Harpagophytum procumbens* DC and *Taraxacun officinale weber*, were analyzed. Standard samples were also analyzed by atomic absorption spectroscopy, in order to have a reference technique for validating the entire procedure.

Keywords: PGMs, Voltammetry, Spectroscopy, Herbal Medicines.

1. INTRODUCTION

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

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

Herbal medicines could appear as not dangerous to health, being derived from natural products. Unfortunately, at now the scientific evidences are not enough to support this general belief. While synthetic products can be purified and stabilized in their formulations, with a defined dosage to obtain the therapeutic effect and minimizing the adverse effects, herbal medicines are difficult to standardize due to raw extracts containing different active ingredients (phytocomplex). This is mainly because different pharmacologically active ingredients are present in the phytocomplex, often similar to synthetic compounds but potentially showing a greater number of adverse effects [1-3].

In the specific case of PGMs, the major problem is linked to their long persistence time in the atmosphere which can lead to a high level of bioaccumulation in the environmental matrices (plants, terrestrial and aquatic organisms). This appears to be one of the possible main causes of serious damage to food safety, as well as one of the preferential routes, in addition to inhalation, of exposure of human beings (as final consumers of the supply chain). In fact, in the human organism there are metabolic processes capable of converting these xenobiotics into non-toxic forms which are subsequently excreted. As long as the absorption rate (even if integrated through dietary sources and herbal medicine) is lower than the conversion and excretion rate, the phenomenon of bioaccumulation will not be observed and the risk of toxicity phenomena will be reduced. The opposite case is found when the speed of absorption is greater than the speed of its excretion and detoxification. In this case, the phenomenon of bioaccumulation is generally observed with consequent exceeding of the "critical" threshold, and the compounds will begin to bind in the sites where they will interfere with normal metabolic functioning. The major risk currently lies in the fact that PGMs, although still considered as micronutrients (and for this reason not fully investigated in terms of toxicological profiles), at high concentrations may express potential toxicity (in all their different bioavailable forms) [4]. For this reason, monitoring and quality control processes in the food supply chain is even more imperative, especially in the sector that includes herbal medicines and food supplements, which is poorly monitored and regulated. Another element not to be overlooked is that, to date, there is no real regulation for the production of herbal medicines, combined with the fact that they can be purchased without a prescription. In light of these regulatory deficiencies, international organizations like WHO (World Health Organization) and FDA (US Food and Drug Administration) simply recommend to check for the quantity of toxic heavy metals in medicinal plants, starting from raw materials. Only for some of these metals the upper-threshold admissible limits are defined as mg L^{-1}/µg g^{-1} for mercury (0.1), lead (3.0), cadmium (1) [5-6]. For all other metals, a real decision has not been evaluated and/or taken, yet.

What so far discussed should fully fall within the pharmacovigilance, i.e. the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem. Unfortunately, pharmacovigilance has been introduced and designed mainly for synthetic drugs. In the field of herbal medicines, where the market develops (often in an uncontrolled way) especially through products that do not require a prescription (even with mail order and internet sales) and considering the possible problems related to public health deriving from uncontrolled products and/or regulated with regard to metals and upper-threshold admissible limits, it is increasingly evident that research in this area plays a fundamental role in safeguarding human health [7].

In the analysis of metals, the most widely used instrumental technique is certainly the electrothermal atomic absorption spectroscopy (ET-AAS) [8-10], especially in the configuration that envisages the graphite furnace (GF-AAS) as atomization device. In the literature there are some works [11, 12] in which the herbal medicine fingerprint is obtained using this technique, which can then be processed using chemometric approaches to investigate their origin [13], or the possible investigation of frauds [14].

In recent years, a line of research has been expanded in our laboratories that includes investigating new approaches to quantitatively determine PGMs in environmental and food samples [15-28] through the application of spectroscopic techniques, and only recently to quantify toxic metals in matrices characterizing this sample categories [9, 29].

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

2. EXPERIMENTAL

2.1 Instrumentation

The acquisition of the voltammograms was performed by a Multipolarograph AMEL Mod. 433 (Milan, Italy). The measuring cells (three-electrode set-up) is structured in the case of iridium with a working electrode consisting in a glassy-carbon electrode (GCE, AMEL, Milan; 7.065-mm^2surface area). For all the other PGMs herein considered, a stationary HMDE was used. In all cases, Ag│AgCl│$KCl_{satd.}$ was the reference electrode and the auxiliary electrode was a platinum-wire electrode. Several rinses of the Teflon voltammetric cell were performed before carrying out measurements, in order to prevent contamination. This procedure was performed as follow: *i)* a first rinsing with supra-pure HNO_3 69%$_{w/w}$, diluted 1:1 with water obtained by a Milli-Q deionizing system (Millipore, Darmstadt, Germany); *ii)* the cell was accurately rinsed by Milli-Q water.

Disposable plastic tips were used for the standard additions. The solutions were previously thermostated at 20.0±0.5°C and treated by pure N_2 for 5 min to remove oxygen. During the measurements, solutions were kept under nitrogen. The magnetic stirring (with Teflon-coated magnetic stirring bar) was constantly maintained during the purge step. For the analyses here presented, the supporting electrolytes and the voltammetric parameters are reported in **Table 1**.

An Atomic Absorption Spectrometer by Perkin-Elmer (Mod. A-Analyst 100 with deuterium background-corrector, Autosampler AS-72 and graphite furnace model HGA 800), was used for the acquisition of ET-AAS measurements. The light sources were Lumina hollow-cathode lamps for single-element measurements (Perkin-Elmer, USA), while the steps of ashing and atomization (different from element to element) were set up as reported in the literature [30]. The instrument settings used were those suggested by the Manufacturer [31] with minor changes.

2.2 Reagents, Reference Solutions, and real samples

Chemicals at suprapure-grade (Merck, Darmstadt, Germany) were used in this work, while a Milli-Q system was use to obtain demineralized water. Reference standard solutions were obtained starting from acidic standard solutions: Ir(III), Pd(II), Pt(II), Rh(III), Ru(III) (1000 mg L^{-1}, Merck, Darmstadt, Germany). Osmium tetroxide solution in water (4%$_{w/w}$, Sigma-Aldrich, Darmstadt, Germany) was used as starting standard solution. In order to optimize the instrumental parameters, two reference materials were used as standard: NIST-SRM 1570a (Spinach Leaves) and NIST-SRM 1573a (Tomato Leaves) (National Institute of Standards and Technology, Gaithersburg, MD, USA). The real samples of *Eucalyptus globulus*, *Harpagophytum procumbens DC* and *Taraxacun officinale weber* analyzed by the herein reported procedure were sold on the local market (Portomaggiore, Italy).

2.3 Sample Preparation

The standard reference materials (NIST-SRM 1570a and NIST-SRM 1573a) and the herbal medicines (*Eucalyptus globulus*, *Harpagophytum procumbens DC* and *Taraxacum officinale Weber*) were solubilized by an acidic attack using a mixture of HCl, HNO_3 and H_2SO_4 (3mL:7mL:5mL, respectively).

The vegetables were accurately weighed (order of magnitude: 1 g), and put in a 25-mL Pyrex-tube for digestion, and a solution containing 4 mL HNO_3 69%$_{w/w}$, 4 mL HCl 37%$_{w/w}$ and 5 mL H_2SO_4 96%$_{w/w}$ was added. Then a Vigreux column condenser was connected to the tube , and the tube was inserted into a cold block digester (appositely homemade); finally, the temperature was progressively raised up to 150°C and maintained at this final temperature for 2 h to achieve the mineralization. After this step, the content was dried, cooled, and dissolved in Milli-Q water (25 mL) or in 25 mL of the supporting electrolyte.

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

For better readability of the text, from this point on the molar concentration (mol L^{-1}) is indicated with M.

The voltammetric procedure for Pd(II), Pt(II) and Rh(III) comprises two steps: *i)* 10-mL sample aliquot of 0.15 M HCl + $1.9 \cdot 10^{-4}$ M dimethylglyoxime (DMG) aqueous reference solution or solutions obtained in the mineralization step were transferred into the voltammetric cell; de-aeration was achieved by bubbling water-saturated pure N_2 for 5 min. The determination of Pd(II) was carried out by SWAdSV; *ii)* 0.5 mL of $1.37 \cdot 10^{-2}$ M formaldehyde + $2.63 \cdot 10^{-2}$ M hydrazine (formazone complex) in 0.15 M HCl solution (final concentration: formaldehyde = 0.65 mM and hydrazine = 1.25 mM) were transferred in the cell. Pt(II) and Rh(III) were determined by SWAdSV.

2.5 2.5 Possible, even if unlikely, interference from Pb(II) and Zn(II) in quantifying Pd(II) and Pt(II) by voltammetry

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

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

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

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

i) 10-mL sample aliquots of 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$ aqueous reference solution, or of solutions obtained in the mineralisation step containing 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$, were transferred into the cell; de-aeration was kept for 5 min by bubbling water-saturated pure N_2. The determination of Os(VIII) and Ru(III) was performed by SWCV with HMDE electrode

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

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

Cu(II) (-0.169±0.015 V *vs.* Ag|AgCl|$KCl_{satd.}$) and Pb(II) (-0.327±0.010 V *vs.* Ag|AgCl|$KCl_{satd.}$) in 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$ supporting electrolyte could be strongly interfering species in the determination of Os(VIII) (-0.243 ±0.010 V *vs.* Ag|AgCl|$KCl_{satd.}$) and Ru(III) (-0.023 ±0.015 V *vs.* Ag|AgCl|$KCl_{satd.}$). This phenomenon, reported in **Figure 2a**, is decidedly important also related to the fact that Cu(II) and Pb(II), in general, are ubiquitary in all real matrices, especially the environmental ones, also at low levels of concentration. This drawback can be avoided using the well-known ability of Ethylene Diamine Tetraacetic Acid (EDTA) to shift peaks toward cathodic potentials if added to the voltammetric cell. In this work, Cu(II) and Pb(II) interfering peaks were shifted by adding EDTA di-sodium salt, specifically: 200 μL EDTA-Na_2 $9.6 \cdot 10^{-3}$ M were added to a 10-mL sample aliquot, obtaining $1.88 \cdot 10^{-4}$ M as final EDTA-Na_2 concentration. Indeed, the EDTA-Na_2 presence results in a shift of potential values in the cathodic direction, either for Cu(II) and Pb(II) or Os(VIII) and Ru(III) [Cu(II): -0.301±0.015; Pb(II): -

0.751±0.010; Os(VIII): -0.096±0.015; Ru(III): -0.529±0.015 V *vs.* Ag|AgCl|$KCl_{satd.}$]. Under these conditions, the final position of the four peaks relevant to these elements makes it possible to achieve complete resolution (see **Figure 2b**), and consequently the quantitative determination becomes possible.

As for Ir(III), it seems that the presence of EDTA-Na_2 slightly changes the position [Ir(III) peak potential with EDTA-Na_2 $1.88 \cdot 10^{-4}$ M: +0.623±0.015 V *vs.* Ag|AgCl|$KCl_{satd.}$; Ir(III) peak potential without EDTA-Na_2: +0.609±0.010 V *vs.* Ag|AgCl|$KCl_{satd.}$], keeping the peak height practically unchanged, and without interference peaks. Following these experimental observations, several elements can be smoothly determined by the procedure suggested here.

3. RESULTS AND DISCUSSION

3.1 Aqueous Reference Solutions

The calculation of limits of detection (LOD) and limits of quantification (LOQ) (**Table 2**) was performed according to IUPAC by the "3 σ approach", were σ is the observed standard deviation. LODs were calculated in the aqueous reference solution and in the digestates obtained from standard reference materials. LODs 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 the slope of the analytical calibration function. Similarly, the LOQs were evaluated as $(K\ s_{y/x})/b$ (K=6).

When voltammetry was used, LODs were obtained directly in the real matrices (**Table 2**); in fact, the standard addition method was applied to create the analytical calibration model. For each analyte, linearity was explored through regression models of voltammetric signal *vs.* metal concentration in aqueous reference solution with the instrumental details reported in **Table 1**. The linearity of the model was demonstrated in the range from LOQs up to 10.0 µg L^{-1}. Determination coefficients R^2 were always higher than 0.9989.

3.2 Quality control and quality assessment

Standard reference materials of Spinach Leaves and Tomato Leaves were analysed by means of voltammetric and spectroscopic method for validating the whole method through the evaluation of its accuracy (**Table 3**). Furthermore, the repeatability was evaluated as relative standard deviation (s_r%) [32-33], from 5 independent measurements, and always resulted better than 6%. Trueness was evaluated through the relative error (e%), and resulted to be on the order of 5-7%. These experimental values observed for the method correspond to satisfactory accuracy.

3.3 Practical applications

Since the quantification of PGMs in reference materials gave good accuracy, the methods were applied to real samples: herbal medicines sold on the local market, and based on *Eucalyptus globulus*, *Harpagophytum procumbens DC* and *Taraxacun officinale weber*. The section "Sample Preparation" describes how samples were treated, after a preliminary step of powdering, by lyophilisation and dried at 80°C for 24 h. **Table 4** reports the experimental results obtained from these commercial herbal medicines in terms of mean concentration values obtained by applying the herein proposed and validated procedure (both voltammetry and spectroscopy).

As can be seen from the results, the two applied techniques provide absolutely comparable quantitative results and represent an excellent starting point for monitoring and quality control on raw materials used in the herbal medicine sector. It should also be noted that the PGMs detected in the herbal medicines considered herein show high levels for Pt(II) and Pd(II) (about 20-30 $\mu g\ g^{-1}$), while Ir(III) is shown to be the least abundant (about 2-5 $\mu g\ g^{-1}$). Intermediate values are instead observed for Rh(III), Os(VIII), and Ru(III) (about 7-15 $\mu g\ g^{-1}$). These values, considering the anthropogenic activities, account for the trend of transition from platinum, palladium and rhodium-based catalysts to the increasing use of iridium, ruthenium and, above all, osmium [1].

In addition, when compared with the average values estimated at the lithosphere level (0.015 $\mu g\ g^{-1}$ for palladium, 0.0001 $\mu g\ g^{-1}$ for rhodium, 0.0001 $\mu g\ g^{-1}$ for ruthenium, 0.005 $\mu g\ g^{-1}$ for osmium, and 0.001 $\mu g\ g^{-1}$ for iridium [34]) it is well understood how their bioaccumulation can lead to serious problems regarding public health and the importance of evaluating the inclusion of upper -threshold admissible limits, just as was done for mercury and other metals. All these elements must in fact be correlated, evaluated, and regulated not only considering these metals as micronutrients, but also evaluating their potential harmfulness when they are assumed in large quantities not only from the surrounding environment, but also as a result of the wide use that nowadays makes supplements and herbal medicines that are easily accessible to anyone and without prescription.

3.4 Comparison between spectroscopic and voltammetric measurements

The results shown in **Tables 3** and **4**, related to the accuracy (precision and trueness) and the real sample analyses, can be considered as an additional validation of the analytical method, based on voltammetry, herein developed. Such a validation can be deduced from the good agreement between the spectroscopic and voltammetric results. Specifically, as regards precision, trueness and detection limits, no significant difference was observed between results obtained with the two different techniques, and analytical performance was good in all cases. Indeed, spectroscopy was taken as reference technique due to its analytical qualities: a well-established and tested robust procedure, suitable for this purpose [35]. However, voltammetry shows interesting advantages with respect to atomic absorption spectroscopy atomic: almost always, it allows the simultaneous determination of many metals (multi-analytes procedure) in a very easy, rapid and inexpensive way. In particular, the standard addition method, which may be easily applied in the herein proposed voltammetric procedures, makes these procedures very rapid, even for multi-element determinations in complex matrices, without renouncing to good selectivity and good sensitivity. For the sake of correctness, it must be highlighted that both Inductively Coupled Plasma (ICP) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) also allow for multi-element analyses, even if ICP-based spectroscopic techniques require time-consuming and expensive sample pre-treatments and enrichment steps, like solvent extraction. Moreover, the instrumentation itself and its maintenance are very expensive (e.g. up to 25-30 times with respect to voltammetry), especially when multi-element spectroscopic determinations are performed.

CONCLUSIONS

As highlighted in this work, the method herein developed and validated represents a valid procedure for the quantification of PGMs in samples of natural origin. Particularly important is its effective application to natural derived products such as herbal medicines (*Eucalyptus globulus*, *Harpagophytum procumbens DC* and *Taraxacun officinale weber*) for which there is currently no precise legislation regarding the maximum content of heavy metals, especially for those relating to the group of Platinum. The procedure described here can be a valid starting point for future applications aimed at standardizing and quality control of these products increasingly used today. This is all the more evident from the application of this procedure to certified reference materials, through which it was possible to obtain important information about the potential of the method. The analytical performances show how a simple sample preparation can be used in order to avoid interferences and obtain reproducible and robust results through the application of voltammetric techniques well known and widespread in the various laboratories.

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CONFLICT OF INTEREST

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

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FIGURE CAPTIONS:

Figure 1. SWAdSV. a) determining Pd(II) in the presence of Pb(II) in *Taraxacun officinale weber*. Supporting electrolyte: 0.15 M HCl + $1.9 \cdot 10^{-4}$ M DMG. Pd(II) corresponds to Peak 1; Pb(II) corresponds to peak 2 ; b) determination of Pt(II) and Rh(III) in the presence of Zn(II) in *Taraxacun officinale weber*. Supporting electrolyte: 0.15 M HCl + $1.9 \cdot 10^{-4}$ M DMG + 0.65 mM formaldehyde + 1.25 mM hydrazine (formazone complex in 0.15 M HCl). Peak 1 corresponds to Zn(II), peak 2 corresponds to Pt(II), peak 3 corresponds to Rh(III). Experimental conditions: see Table 1

Figure 2. SWCV. Os(VIII) and Ru(III) in presence of Cu(II) and Pb(II) in *Taraxacun officinale weber*. Supporting electrolyte: 0.5 M acetate buffer pH 4.9 + $7.3 \cdot 10^{-2}$ M $NaBrO_3$ in absence (a) and presence (b) of EDTA-Na_2. Peak 1 corresponds to Os(VIII), peak 2 corresponds to Cu(II), peak 3 corresponds to Ru(III), peak 4 corresponds to Pb(II). Experimental conditions: see Table 2.