Validation of an Electrothermal Atomization Atomic Absorption Spectrometry Method for the Determination of Aluminum, Copper, and Lead in Grapes

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An electrothermal atomization atomic absorption spectrometry method was validated to quantify aluminum, copper, and lead in grapes. The limits of detection were 1.11, 0.19, and 0.35 μg/L for Al, Cu, and Pb, respectively. The linearity ranges under optimized conditions were 1.11−50.0, 0.19−25, and 0.35−50.0 μg/L for Al, Cu, and Pb, respectively. The limits of quantification were 74.0, 12.5, and 11.6 ng/g of dry weight for Al, Cu, and Pb, respectively. For all of the metals, the precision for the instrumental method was lower than 5.4% and for the analytical method, lower than 10%. The accuracy of the method was evaluated by the standard additions method, the recoveries being higher than 90% for all of the concentrations added. An interference study was also carried out in a simulated matrix, and it was verified that the deviations from the expected values were lower than 3.4% for all of the metals. The method was applied to the monitoring of the metals referred to above in 35 samples of grapes obtained in marketplaces and at farmhouses. The metals were quantified in the whole grapes, washed or not, and in the peel and pulp of unwashed grapes.

KEYWORDS: Method validation; atomic absorption spectrometry; metal residues; grapes

INTRODUCTION

Grapes constitute a very important fraction of fruit production the world over, destined to both consumption as such, namely, as table grapes, and production of wine of several different characteristics. The quality of the grapes can be considered in several aspects, but their nutritional value and absence of contaminants are among the principal determinants.

Heavy metals can be present in grapes mainly as a result of the type of soil (endogenous contamination) or exogenous deposition resulting from the air contamination and the application of mineral pesticides.

In fact, vineyards are commonly attacked by fungal diseases, and the grapevines are normally treated with several pesticides, in their different life periods, including very close to the maturation and the harvest, the remaining residues in the fruit being an important point to be considered. Some of these pesticides can have in their constitution metals such as copper, which, being an essential element, can also raise toxicological concerns when at excessive levels (*1*).

Several other metals raise toxicological concerns, lead being one of the most monitored in food because it is so widespread in the environment in spite of restrictive international measures, among which the most important being, undoubtedly, the prohibition of leaded gasoline. This measure was very efficacious in reducing the ingestion of lead by human and animals; as a result, food products of vegetal origin are continuously less and less contaminated by this metal. Due to other origins of lead in the environment and its recognized hazardous effects (*2*) and the tendency to lower the allowable levels in food by international organizations, the interest in its control in this fruit goes without question.

Aluminum is a ubiquitous metal that has raised particular concern recently due to the suspicion of its involvement in Alzheimer's disease. Acidified soils can favor the high uptake of aluminum by plants, contributing to their endogenous levels as well as particle deposition in fruits from polluted atmospheres (*3*). The control of Al levels in food is, consequently, of growing importance and concern.

Portugal, like other southern countries, is a major producer of grapes, both for consumption as fruit and for wine production, some of them unique in the world, examples being Port wine and Vinho Verde wine.

The literature available on methods for the quantification of heavy metals in grapes is scarce and, overall, not fully validated. To our knowledge, only the work of Olalla et al. (*4*) presents a validated method for the measurement of copper and zinc in grapes by atomic absorption spectrometry with a graphite furnace. In the works of Al Nasir et al. (*5*) and Angelova et al. (*6*), lead and copper were quantified in the grapes by flame atomic absorption spectrometry, but data of method validation were not presented.

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Metals in Grapes

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Table 1. Instrumental Conditions and Graphite Furnace Programs for Measurement of Al, Cu, and Pb in Grapes

parameter	Al	Cu	Pb
wavelength (nm)	309.3	324.8	283.3
ashing temperature (°C)	1700	1100	700
atomization temperature (°C)	2500	2300	1800
injection volume of sample/modifier[a] (μL)	15/10		
inert gas	argon		
flow rate (mL/min)	300		
background correction	deuterium arc		
HGA tubes	with integrated platform		
gas stop flow	atomization step		
measurement mode	integrated absorbance		

[a] The autosampler was programmed to pipet sequentially 10 μL of the modifier solution and 15 μL of the digested sample/standard solution and dispense them together on the platform. For Al determination the chemical modifier was 0.01 mg of $Mg(NO_3)_2$. For Pb the chemical modifier was 0.03 mg of $Pd(NO_3)_2$ + 0.02 mg of $Mg(NO_3)_2$. Cu was evaluated without chemical modifier.

The main objective of the present work was to validate a method to accurately quantify aluminum, copper, and lead in grapes and to apply the validated method to their quantification in grapes of several varieties, obtained directly from producers in different Portuguese viticulture regions and also in marketplaces.

MATERIALS AND METHODS

Reagents and Materials. *All solutions* were prepared with doubly deionized water, and the chemicals used (HF, HCl, HNO_3, and H_2O_2) were of pro analysis grade (Merck).

Metal standard solutions were prepared daily from 1000 mg/L solutions (Spectrosol BDH) in 0.2% HNO_3 Suprapure grade (Merck).

Chemical modifiers consisted of 1 g/L of $Mg(NO_3)_2$ solution and 2 g/L $Mg(NO_3)_2$ + 3 g/L $Pd(NO_3)_2$ solution, Suprapur grade from Merck, prepared in 15% (v/v) Suprapur nitric acid.

Certified Reference Material, spinach leaves, 1570a, was supplied by the U.S. National Institute Standards and Technology (NIST).

Decontamination of Material. To avoid contamination of the samples, all PTFE materials (Kartell Teflon vessels, pipets, micropipet tips, and autosampler cups) were immersed in freshly prepared 15% (v/v) pro analysis HNO_3 (Merck) during 24 h, then rinsed thoroughly with doubly deionized water, and dried in a dust-free area before use.

Apparatus. *The water purification system* was a Seralpur PRO 90 CN and Seradest LFM 20.

Balance. All analytical weighings were performed with a Mettler Toledo balance model AB265-S.

The stove was a Heraeus model D-6450.

Spectrophotometer. Metal quantifications were carried out in a Perkin-Elmer HGA-850 furnace installed in a model *A*Analyst 300 spectrometer with deuterium arc background correction, equipped with an AS-800 autosampler and a HP Deskjet 920C. The analyses were performed using Perkin-Elmer HGA tubes with integrated platform. The instrumental operating conditions and furnace programs for the determination of the elements are summarized in **Table 1**.

Sampling. Table grapes were purchased in markets (10 samples), about 1 kg each. Twenty-five samples were obtained from the local producers of several regions of Portugal, being a heterogeneous group of grapes, normally destined to produce wine, at both industrial and domestic scale, but also appreciated as fresh fruit by the local population.

The grapes were stored in plastic bags and conserved at 4 °C during 48 h until analysis pretreatment.

Sample Preparation. From each sample of grapes two portions of 25 berries each were randomly separated. One portion was washed by manual agitation during 1 min with demineralized water. Some berries of the other portion were maintained intact for analysis without washing, and others were subdivided into grape peel and pulp with the grape seeds.

All of the samples were cut into small portions with a plastic knife previously rinsed with 15% HNO_3 and ultrapure water, packed in PVC decontaminated tubes, and placed in a stove at 60 °C for several days. The dried samples were reduced to powder in a closed 50 mL Teflon container, and two digestion procedures were carried out.

Wet Digestion Procedure A. (a) Approximately 0.5 g (for Al and Cu) or 1.0 g (for Pb) of powdered sample was accurately weighed and transferred to a closed Teflon container which (b) after the addition of 1.5 mL of HNO_3 + 0.5 mL of HCl and 0.25 mL of H_2O_2 (c) was closed for digestion in a thermostatically controlled stove at 90 ± 2 °C overnight for 17 h.

(d) The digested solution was transferred to a decontaminated tube and diluted to 10 mL with doubly deionized water.

Wet Digestion Procedure B. (a) Approximately 0.5 g (for Al and Cu) or 1 g (for Pb) of powdered sample was accurately weighed and transferred to a Teflon container.

(b) Step 1: 0.5 mL of HF and 2 mL of HNO_3 were added to the sample and heated at 90 ± 2 °C during 8 h to enable the volatilization of silicates.

(c) Step 2: To this residue were added 1.5 mL of HNO_3 + 0.5 mL HCl + 0.25 mL H_2O_2, and (d) the Teflon container was closed for digestion during 17 h in a stove thermostatically controlled at 90 ± 2 °C.

(e) The digested solution was transferred to a decontaminated tube and diluted to 10 mL with doubly deionized water. The measurement of the metals was performed in this solution.

Along with each batch digestion sample a set of three blanks was included, consisting of the same proportion of the acid mixture and submitted to the same pretreatment steps.

Validation of the Method. The analytical conditions for metal measurement were established by using the respective standard acid solutions and digested grape sample solutions prepared according to procedure A digestion.

(a) *Calibration* against standard acid solutions was performed, and the linear ranges were established for each element using working ranges from 0 to 50.0 μg/L for Al, from 0 to 25.0 μg/L for Cu, and from 0 to 50.0 μg/L for Pb.

Every day the standard working solutions were freshly prepared and analyzed to guarantee the precision of the method.

(b) To calculate *the detection limit of the instrumental measurement*, 20 determinations were carried out on a 0.2% HNO_3 solution, the value calculated as $3s/m$, where s is the standard deviation of the blank measurements and m is the slope of the calibration curve.

(c) The *limit of quantification* was calculated as $10s/m$, the blank being a 0.2% HNO_3 solution.

(d) *Instrumental precision* was evaluated by measuring the absorbance signals in the same digested grape sample 20 times under the established instrumental conditions.

(e) For evaluation of the *precision of the analytical method,* estimated as within-batch, readings of 20 different digested solutions of the same grape sample were performed for all of the analytes.

(f) The *recovery studies* were performed by the standard additions method. A sample of whole grapes was divided into several aliquots. In one aliquot the concentrations of Al, Cu, and Pb were measured (initial concentration). To other aliquots were added four different concentrations (between 5 and 50.0 μg/L for aluminum and between 2.5 and 25 μg/L for copper and lead) of standard metal solutions (six replicate portions for each concentration), the final concentrations of the metals in the spiked samples were determined, and the respective recoveries were calculated.

(g) The *interference studies* of the matrix were carried out in a simulated matrix prepared by mixing the principal organic and inorganic constituents of grapes, which are listed in **Table 2** (*7*). Four concentrations of each metal were added to several aliquots of this simulated matrix (between 5.0 and 50.0 μg/L for aluminum, between 2.5 and 20.0 μg/L for copper, and between 2.5 and 25 μg/L for lead). These spiked matrices were submitted to the overall procedure, the metals were measured in the digested solutions against the respective standards, and the deviations of the expected values were determined.

(h) Although for grapes there was no available certified reference material, the accuracy of the method was evaluated by analyzing

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Table 2. Principal Constituents of Grapes Used To Prepare the Simulated Matrix To Perform the Interference Studies

inorganic constituent	concentration (μg/g)	organic constituent	concentration (mg/g)
chloride	0.02	carbohydrate	171
calcium	180	fat	1.0
potassium	1920	protein	5.0
sodium	210	glucose	74
phosphorus	0.20	fructose	75
magnesium	93	sucrose	4.5
iron	5.1		
manganese	0.73		
cobalt	0.014		
zinc	0.82		
nickel	0.08		
chromium	0.02		
silicon	0.003		

Table 3. Deviations from Expected Values for the Metals Obtained after Wet Digestion without and with HF (Levels Are Expressed as Mean Values of 10 Independent Assays)

	Al (μg/g)	Cu (μg/g)	Pb (μg/g)
procedure A[a]	4.48	2.22	0.193
procedure B[b]	4.12	2.10	0.177
deviation (%)	−8.7	−5.7	−9.0

[a] Procedure A, wet digestion with HNO_3 + HCl + H_2O_2. [b] Procedure B, wet digestion with HF + HNO_3 followed by HNO_3 + HCl + H_2O_2.

certified reference material for spinach leaves, 1570a, supplied by the U.S. National Institute of Standards and Technology (NIST). For this purpose, from the powdered reference material, 12 aliquots of matrix were submitted to the proposed mineralization procedure and absorbance readings were obtained using the established instrumental conditions.

Application of the Validated Method. The validated method was applied to the quantification of aluminum, copper, and lead in the digested samples of whole grapes (water washed and unwashed), unwashed grape peels, and pulp of unwashed grapes.

RESULTS AND DISCUSSION

Digestion Procedure. The quantification of trace elements in biological samples generally implies the simplification of the matrix to obtain the effective dissolution of the samples.

The principal steps of the sample simplification (whole grape, peel, and pulp) consisted of dehydration in the stove at 60 °C for several days, pulverization of the dried product in a Teflon container, and addition of an oxidant mixture (HNO_3 + HCl + H_2O_2) to mineralize the sample (procedure A). Because silicates can be present in vegetable matrices in great quantities, a digestion procedure including the dissolution with HF was also tested to evaluate the putative interference of the silicates in the measurement of the metals, especially in aluminum (procedure B). The concentrations of the three metals in the digestion solutions obtained by both procedures were evaluated and compared and no significant statistical differences detected ($\leq$9%) (see **Table 3**); thus, procedure A was adopted because it is simple and efficacious, originating a complete digestion without residue.

Validation of the Method. The *linearity ranges* evaluated by analyzing standard solutions of the metals under the optimized conditions were set between 1.11 and 50.0 μg/L, between 0.19 and 25 μg/L, and between 0.35 and 50.0 μg/L for Al, Cu, and Pb, respectively (see **Table 4**).

The *detection limits of the instrumental measurement* evaluated in blank solutions were 1.11, 0.19, and 0.35 μg/L for Al,

Table 4. Performance of the Method

	precision (CV%)		linearity (μg/L)	detection limit		quantification limit	
	instrumental procedure	overall procedure		μg/L	ng/L	μg/L	ng/g
Al	2.3	9.9	1.11–50.0	1.11	22.2	3.70	74.0
Cu	3.0	9.1	0.19–25.0	0.19	3.8	0.63	12.5
Pb	5.4	6.3	0.35–50.0	0.35	3.5	1.16	11.6

Table 5. Levels of Al, Cu, and Pb Found in Standard Reference Material for Spinach Leaves (SRM 1570a)

	certified value (μg/g ± SD)	found value ($n = 12$) (μg/g ± SD)
Al	310 ± 11	301 ± 4
Cu	12.2 ± 0.6	11.9 ± 0.4
Pb	0.20[a]	0.19 ± 0.03

[a] Value not certified.

Cu, and Pb, respectively. On the basis of 0.5 g (for Al and Cu) or 1.0 g (for Pb) of dried sample in a final volume of 10 mL, the limits of detection were 22.2, 3.8, and 3.5 ng/g and the limits of quantification were 74.0, 12.5, and 11.6 ng/g for Al, Cu, and Pb, respectively, as is hown in **Table 4**.

The *precision* of the method, evaluated under repeatable conditions, both for the instrumental and for the analytical procedures, was lower than 10% for all of the metals, which is an acceptable level of variability of the results (**Table 4**).

Although for grapes there was no available certified reference material, the *accuracy* studies were performed by analyzing other certified reference material (spinach leaves, 1570a), supplied by NIST. For this purpose, 12 aliquots of powdered reference material were submitted to the established digestion method and absorbance readings were obtained using the instrumental conditions summarized in **Table 1**. The certified values in the material were 310 ± 11 and 12.2 ± 0.6 μg/g for Al and Cu, respectively, and the values found by applying the implemented methodology were 301 ± 4 and 11.9 ± 0.4 μg/g for Al and Cu, respectively. For Pb, the content, although not certified, was 0.2 μg/g, and the value obtained by our analytical method was 0.19 ± 0.03. Application of the Student t test at the 95% confidence level indicated significant agreement between the values obtained by applying our method and the certified values (**Table 5**).

The *accuracy* study was also performed by using the standard additions method, submitting the spiked samples to the overall procedure and measuring the metals in the samples digested as described. This study was carried out with four different concentrations of each metal (between 5.0 and 50 μg/L for aluminum and between 2.5 and 25 μg/L for copper and lead). The recoveries obtained were always higher than 90% for the three metals studied, as is shown in **Table 6**.

The results obtained for the precision and the accuracy studies showed that there were neither contaminations nor losses during the pretreatment steps for all of the analyzed metals and the effect of the matrix was efficiently suppressed by the digestion procedure.

The results of the *interferences* study performed in the simulated matrices are summarized in **Table 7**. As can be observed, the deviations from the expected values are very low for all of the metals and for all of the added concentrations, always being lower than 3.4%. From this study we can conclude

Table 6. Statistical Results for the Recoveries Obtained by the Standard Additions Method[a]

	C_1 (μg/L)	C_2 (μg/L)	C_3 (μg/L)	recovery (%)
Al	24.15 ± 2.54	5.0	28.90 ± 0.10	95 ± 2
		10.0	33.65 ± 0.20	95 ± 2
		25.0	48.40 ± 0.75	97 ± 3
		50.0	71.65 ± 2.00	94 ± 4
Cu	14.40 ± 0.29	2.5	16.75 ± 0.075	94 ± 3
		5.0	19.05 ± 0.10	93 ± 2
		10.0	23.80 ± 0.30	94 ± 3
		25.0	38.15 ± 1.00	95 ± 4
Pb	1.95 ± 0.20	2.5	4.37 ± 0.05	97 ± 2
		5.0	6.70 ± 0.10	95 ± 2
		10.0	11.25 ± 0.10	93 ± 1
		25.0	25.45 ± 0.25	94 ± 1

[a] C_1 = initial concentration of Al, Cu, and Pb found in a whole grape sample. C_2 = concentration of standard metal solution added to whole grape samples prior to the application of the overall procedure. C_3 = final concentrations found in the spiked whole grape samples.

Table 7. Deviations from Expected Values for Al, Cu, and Pb Obtained in the Interference Studies

	concentration added[a] (μg/L)	concentration found[a] (μg/L)	deviation from expected value (%)
Al	5.0	4.8	3.4
	10.0	9.5	3.1
	25.0	23.8	1.4
	50.0	47.0	2.8
Cu	2.5	2.4	3.0
	5.0	4.6	2.6
	10.0	9.2	1.6
	20.0	18.4	2.0
Pb	2.5	2.4	3.1
	5.0	4.7	3.1
	10.0	9.2	1.5
	25.0	22.8	1.1

[a] Results are expressed as mean values of six assays.

Table 8. Contents of Al, Cu, and Pb in 35 Samples of Portuguese Grapes of Several Varieties Obtained in Markets and Farmhouses

	μg/g Al, wet weight, mean ± SD	μg/g Cu, wet weight, mean ± SD	ng/g Pb, wet weight, mean ± SD
unwashed grape peel	1.56 ± 0.54 (0.34–6.02)[a]	0.51 ± 0.18 (0.10–2.65)	3.13 ± 1.08 (0.806–7.77)
pulp of unwashed grape	0.41 ± 0.14 (0.15–0.88)	0.38 ± 0.13 (0.14–0.87)	2.14 ± 0.74 (0.739–5.29)
unwashed whole grape	1.90 ± 0.66 (0.49–4.95)	0.86 ± 0.30 (0.32–2.71)	5.13 ± 1.78 (1.74–9.12)
whole washed grape	0.81 ± 0.27 (0.16–2.23)	0.78 ± 0.26 (0.31–2.00)	<1.74 (<1.74–6.84)

[a] The minimum and maximum values are given in parentheses.

that there was no noticeable interference of the principal constituents of the matrix on the analyzed metals.

Application of the Method Implemented. The method was applied to 35 samples of grapes acquired both in the market (table grapes, $n = 10$) and directly from the farmhouses in the principal winegrowing regions of Portugal (table grapes and grapes mainly destined to make wine, $n = 25$).

Table 8 shows the levels, in terms of mean values, of the three quantified metals in the whole grape samples, washed or not, and in the peel or pulp of unwashed grapes. The mean values are expressed in micrograms per gram of wet weight for aluminum and copper and in nanograms per gram of wet weight for lead. Because the study was carried out in the dried samples, the values obtained were converted into the respective wet weight by taking into account the moisture of the different portions analyzed determined during the pretreatment sample procedure, which were 85, 89, and 82% for whole grapes, pulp, and peel, respectively. Also in terms of mean values, the percentage of the pulp and the peel relative to the whole grapes was 60 and 40%, respectively.

Aluminum. The aluminum levels present in whole grapes showed a high dispersion, ranging from 0.49 to 4.95 μg/g, the mean value being 1.90 μg/g. The contents of this element in the whole washed grapes lowered to a half mean value of 0.81 μg/g, the values ranging between 0.16 and 2.23 μg/g. When aluminum was determined in the grape peel, it was verified that the principal contents of 1.56 μg/g were present in this part; 0.41 μg/g is the mean value found in the pulp. The application of the Student t test (at the 95% confidence level) showed that the aluminum content present in the unwashed whole grapes was significantly higher than that present in washed whole grapes as well as were the contents in the grape peel compared to the pulp. We can conclude that the main part of this element present in the grapes is of exogenous origin, as a result of the deposition of dust from the atmosphere, and that about two-thirds of the aluminum does not adhere to the peel because it is removed by the simple water-washing procedure (manual agitation of the grapes during 1 min in demineralized water).

In terms of mean values, aluminum was the metal present in higher concentrations. Due to the growing concern raised by its putative toxic effects on humans, its levels should be monitored in food, the origin of its presence detected, and attempts made for its reduction.

Copper. The content of copper, in terms of mean value, was 0.86 μg/g in whole grapes, ranging from 0.32 to 2.71 μg/g. When the grapes were washed, only 9.3% of the copper was removed. This result was confirmed by the Student t test (at the 95% confidence level), showing that there were no significant differences between washed and unwashed grapes.

In spite of the low efficiency in removing Cu by washing, >50% of the copper was present in the peel (0.51 μg/g, mean value). We can speculate that the copper present in the grapes is essentially of endogenous origin, that is, extracted by the plant from the soil, and is linked to endogenous constituents, which, although in the peel, are not removed by water washing. The capacity of several plant species to efficiently extract metals from the soils, especially copper, is very well documented (*1*). The vineyards are frequently attacked by fungal diseases, and copper formulations are applied as a spray in the treatment. Part of the copper present in the peel can thus result from these applications that, being exogenous, can be sufficiently adherent to the peel and not removed by agitation in water as deposited dust particles are.

Ollala et al. (*4*) found a similar content of copper, 0.52 μg/g, in grapes grown in Spain, although Miller-Ihli (*8*) reported a higher value of 1.62 μg/g in American grapes and Al Nasir et al. (*5*) reported the lowest content of 0.18 μg/g in grapes from Jordan. The discrepancy of the values can be due to agricultural practices and to several environmental factors. This is very well documented in a study performed by Angelova et al. (*6*), in which they verified that grapes grown in an industrially polluted region show copper levels significantly higher than those in grapes grown in a nonpolluted region.

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Lead. The mean level of lead present in the whole grapes was 5.13 ng/g, and the individual values presented a high dispersion, ranging from 1.74 to 9.12 ng/g. In terms of mean values, the lead content in whole washed grapes was reduced to levels lower than the limit of quantification, although the dispersion of the results remains very high. It is not possible to draw conclusions about the origin of lead in the grapes, but the dispersion of the results can be indicative of mixed factors, that is, extraction from the soil (some samples retained the lead after washing) or air pollution (the samples for which the lead content was reduced to values not detectable). These factors also affect the levels of lead in grapes as verified by Angelova et al. (*6*).

Generally, the dispersion of the content values of the metals present in the grapes in our study is not surprising because the grapes were of different varieties, grown in soils with very different mineral constitutions and at vineyards having different environments with regard to road traffic, industrial plants, etc. All of these factors can explain the variability of the obtained results.

To our knowledge, there are no established maximum residue levels for aluminum and copper in grapes; referring to lead, the European Community establishes 0.2 μg/g of wet weight in berries and other small fruits (*9*). This level was not surpassed in any of the analyzed samples, neither in terms of mean values nor in individual values, 9.12 ng/g being the highest value found in an unwashed whole grape sample.

Overall, we can assume that the presence of the three metals studied in the grapes resulted either from fallout from the atmosphere or from penetration via the root into the grapevine, accumulating in different parts, including in the fruits.

The analytical method proposed consisting of wet acid digestion of the samples and metal determination by atomic absorption spectrometry with electrothermal atomization is suitable for the quantification of aluminum, copper, and lead in grapes at residual levels. It presents very low limits of quantification and is precise and accurate, enabling the control of the three metals with excellent efficiency.

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