

DEVELOPMENT AND VALIDATION OF A MODIFIER-FREE METHOD FOR THE DETERMINATION OF CADMIUM AND LEAD IN ORANGE JUICE SAMPLES

Muhammad Abbas Bhutto, Faiza Anwar*, Uzma Sitara, Riaz Uddin and Parwaiz Ahmed Baloch

PARC-Food Quality & Safety Research Institute, Southern-zone Agriculture Research Center University of Karachi.

*Corresponding author's email: faizaanwar@parc.gov.pk

ABSTRACT

A method for determining Cadmium (Cd) and Lead (Pb) was developed and validated using ICH (International Council for Harmonisation) guidelines in canned/fresh orange juice. The analysis was executed on Varian's GFAAS without the application of a modifier using a hollow cathode lamp (HCL) and a deuterium (D2) lamp. The wavelength and slit width of 217.0nm and 0.5nm were selected for lead while 228.8nm and 1.0nm for cadmium. The method's efficiency was assessed by conducting the system suitability parameters. It was discovered that the limits of detection (LOD) for Cd and Pb were 0.052 and 0.16 µg/mL with a limit of quantification (LOQ) of 0.159 and 0.51µg/mL respectively. The intraday precision for Cd and Pb was estimated to possess a relative standard deviation (RSD) of 1.89% and 1.43%, with an inter-day precision of 2.45% and 2.16% respectively. Accuracy of the proposed method showed percent recovery of 100%, 92%, and 108% for Cd and 90%, 102% and 100% for Pb. The analytical method was found accurate, precise, effective, and appropriate for the quantitative determination of Cd and Pb in fresh/canned orange juices.

Key-words: Method validation, Cadmium, Lead, Graphite Furnace, Modifier

INTRODUCTION

Atomic Absorption Spectrophotometry (AAS) coupled with a Graphite tube atomizer (GTA) is the technique used widely for determining metals in foods and matrices due to its excellent selectivity and sensitivity. Nonetheless, in graphite furnace AAS, modifiers are frequently used to improve analytical performance and restrict internal systematic and analyte interferences (Atasoy, 2023). However, modifiers emit restrictions limitation linked with each of their efficiency in complex matrices, temperature-dependent therefore are incompatible with all types of samples. Most important it may interact with other sample analytes causing bias in the analysis. Therefore, the practice of modifier utilization restricts all type interferences during the analysis of metals even at trace levels while using graphite furnace atomic absorption spectrometry (GFAAS) (Liu *et al.*, 2023). The use of a defined modifier transforms the target analyte into a phase of higher thermostability and enhances its pyrolysis temperatures to remove analytical biases during thermal pretreatment without disturbing the analyte prior to the atomization stage (Patriarca *et al.*, 2021; Bolea-Fernandez *et al.*, 2024).

Commonly used modifiers for lead and cadmium are ammonium phosphate, magnesium nitrate, nitric acid, ascorbic acid, Triton X-100, DDTC (Diethyldithiocarbamate), APDC (Ammonium Pyrrolidine Dithiocarbamate) and Palladium, respectively (Subramanian, 1996; Evans *et al.*, 2001; Fisher *et al.*, 2003; Evans *et al.*, 2007). However, an effective matrix modifier significantly minimizes spectral interferences by reducing absorption and scattering caused by concomitant molecular or atomic lines. This enhances volatilization, eliminates vapor-phase interferences, and plays a crucial role in the stabilized temperature platform furnace (STPF) concept for GFAAS. (Zong, 1996; Welz *et al.*, 2019). Among the reagents, Volynsky, (2000) proposed several modifiers such as the use of Ni to lower the volatility of (As) and (Se); ammonium phosphate to stabilize cadmium; ammonium nitrate with brackish water samples for removal of NaCl at reduced pyrolysis temperatures. Other modifiers are as well as confirmed by several scientists in recent years were (Mo), (La), (H₃PO₄), Mg(NO₃)₂, (K₂Cr₂O₇), and several others (Schlemmer and Welz, 1986; Wierzbicka *et al.*, 2022). While using GFAAS-specific requirements, several indispensable instructions in routine work should be considered which include: stabilizing the analyte at the highest possible pyrolysis temperature, using a high-purity modifier suitable for multiple elements, avoiding high concentrations of elements analyzed later, minimizing graphite tube wear, and preventing excessive background interference near the analyte's wavelength (Ortner *et al.*, 2002; Beauchemin, 2008; Wheal *et al.*, 2011). However, most of the suggested modifiers may not confirm one or all of these expectations. Nickel, often recommended for As, Au, Bi, Sb, Se, and Te analysis, is unsuitable due to its frequent low-level determination; copper in replacement of Ni is proposed (Xu and Liang 1997; Feo *et al.*, 2003). Pure Ammonium phosphate modifier recommended for Ag, Cd, Pb, and Sn is unavailable (Schlemmer and Welz, 1986). An investigation by Volynsky (2004) revealed that

in the presence of palladium or platinum, inorganic and organic Tellurium (Te) could be heated to 1050°C. Palladium may be used with pyrolysis temperature of up to 1200°C in the analysis of Bismuth (Bi) in the matrix like wastewater, seawater, and urine, (Qiao, 1992).

Analytical techniques for measuring metals in liquid samples differ primarily in analyte detection and atomization sources. ICP-MS determines metals by measuring the m/z ratio of analyte ions (Khadim et al., 2024), while ICP-OES detects metals via optical emission at specific wavelengths (Senila, 2024). Atomic spectrometric techniques rely on radiation absorption at characteristic wavelengths, with atomization achieved through graphite furnace (GF-AAS) or flame (F-AAS) methods (Butcher, 2024). For volatile metals like mercury, thermal desorption allows direct measurement in samples (Gustin *et al.*, 2024). Currently, the GF-AAS technique is commonly recognized due to its high selectivity and sensitivity. The significance of analytes such as Cd and Pb in liquid juice samples through GFAAS without applying modifiers necessitates validation of the developed method. The present study therefore aimed to develop and validate a novel analytical technique for assessing Cd and Pb in canned juice through GFAAS without application of any modifier.

MATERIALS AND METHODS

The reagents and chemicals (HNO₃, HClO₄, and H₂SO₄), The Cd and Pb standards used in the experiment were of HPLC grade and were purchased from Merck in Darmstadt, Germany. For the preparation of every solution, deionized water was utilized. A Varian's Atomic absorption spectrophotometer (220FS) coupled with a Graphite Tube Atomizer (GTA) was used for experimental work. To cool down the system during an experiment, a chiller of Affinity Model # RAA-003B-BE01CB was used. Argon gas (99.99% pure) was used for a better signal response. The operating system Windows 98 with installed software of "SpectrAA" at a wavelength of 228.8nm for the analysis of Cd and 217.0nm for Pb was used. Other operating details are provided in Table 1 and Table 2.

Sample preparation

Digestion of Samples was prepared by taking 50mL test material fruit juice (Orange), which was received from FAPAS, UK. Ten samples of 5.0mL exactly were taken into a 50 mL ceramic crucible and dried in the furnace at 120 °C approximately for 12 hours. When samples cooled down, 0.5 mL of sulfuric acid was added to each crucible and again kept in a muffle furnace for 12 hours and the temperature was increased up to 500 °C until white ash was obtained. The residues were moistened with a few drops of distilled water and 0.5mL of concentrated nitric acid and were kept in the furnace for 45 minutes at 500 °C. Crucibles were cooled down at room temperature in a desiccator and ash was dissolved with 10mL of 2M HCl, gently heating on the hot plate. The solution then was cooled at room temperature and quantitatively transferred into a 25mL volumetric flask and volume was raised up to the mark with 2M HCl. The same treatment was used for the preparation of blanks. This long sample treatment was necessary for slow dry ashing to prevent volatilization losses.

Calibration standard preparation

For linearity, calibration standards were prepared with standard Cd and Pb solution of (Merck 1000mg/L) in the range of 0.005–0.05 µg/mL for Cd and 0.03–0.18 µg/mL for Pb in 25mL volumetric flask and the volume was made up by 2M HCl.

Table 1. Atomic absorption Spectrophotometer parameter for Cd and Pb.

Parameters	GTA	
	Cd	Pb
Concentration Unit	µ g / L (ppb)	µ g / L(ppb)
Measurement mode	Peak Height	Peak Height
Wave Length (nm)	228.8	217.0
Slit Width (nm)	0.5	1.0
Lamp Current (mA)	4.0	10.0
Background Correction	On	On
Sample volume (µ L)	15	15

Table 2. Graphite tube atomizer (GTA) parameters for Cd and Pb.

Step	Temp(° C)	Times (s)	Flow (L/min)	Gas type	Read	Signal storage
1	85	5.0	3.0	Normal	No	No
2	95	40.0	3.0	``	No	No
3	120	10.0	3.0	``	No	No
4	400	5.0	3.0	``	No	No
5	400	1.0	3.0	``	No	No
6	400	2.0	0.0	``	No	Yes
7	2100	1.0	0.0	``	Yes	Yes
8	2100	2.0	0.0	``	Yes	Yes
9	2100	2.0	3.0	``	No	Yes

RESULTS AND DISCUSSION

Various parameters were assessed to validate the analytical method for quantifying heavy metals (Cd and Pb) in fresh orange juice. i.e. system suitability, linearity, accuracy, and precision.

System suitability

This test evaluates system performance, with precision in replicate results confirming its suitability for the proposed method. It was assessed through ten consecutive analyses of the same standard i.e. 0.3 µg/mL for Cd and 0.4 µg/mL for Pb and their response were recorded (Table 3). The precision for both metals was found (%RSD <5) within the acceptable range.

Table 3. System suitability test for cadmium (Cd) and Lead (Pb).

Sr No.	Absorbance(Cd)	Absorbance(Pb)
1	2.53	1.31
2	2.41	1.41
3	2.43	1.41
4	2.51	1.33
5	2.57	1.36
6	2.45	1.40
7	2.49	1.32
8	2.41	1.31
9	2.53	1.33
10	2.52	1.31
Average	2.48	1.34
SD	0.05	0.04
%RSD	2.15	3.01

Linearity

Linearity for Cd and Pb was evaluated by plotting a calibration curve of absorbance versus concentration (ranging at 0.05–0.5 µg/mL for Cd and 0.03–0.18 µg/mL for Pb (Fig 1 and Fig 2). All calibration curves had regression coefficients above 0.99, confirming strong linearity. (Table 4).

Table 4. Regression Characteristics and Sensitivity of the Method.

	Linearity*	Slope	R ²	Intercept	LoD*	LoQ*
Cadmium	0.005–0.05	9.72	0.991	-0.018	0.052	0.159
Mercury	0.03–0.18	0.1605	0.992	-0.001	0.16	0.51

* (µg/mL⁻¹)

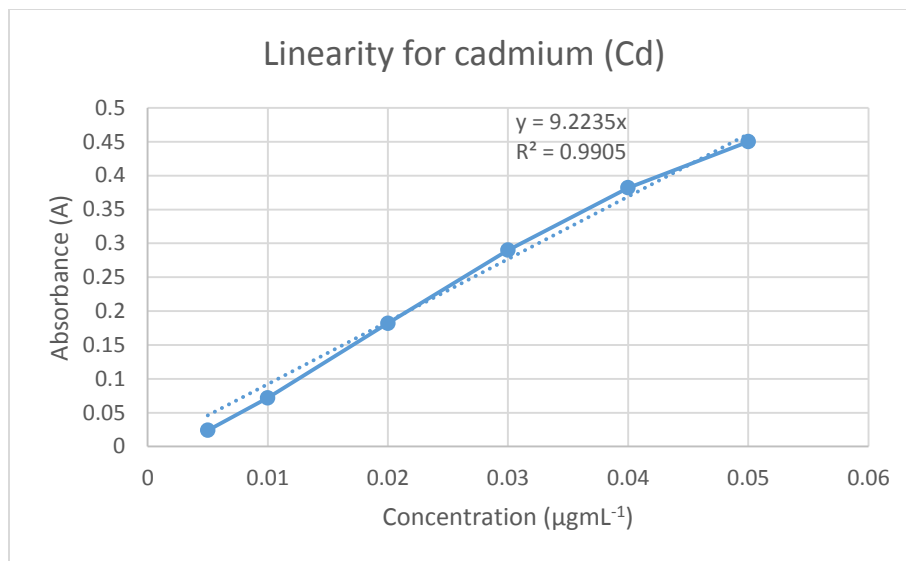


Fig 1. Calibration curve between absorbance and concentration for Cadmium (Cd).

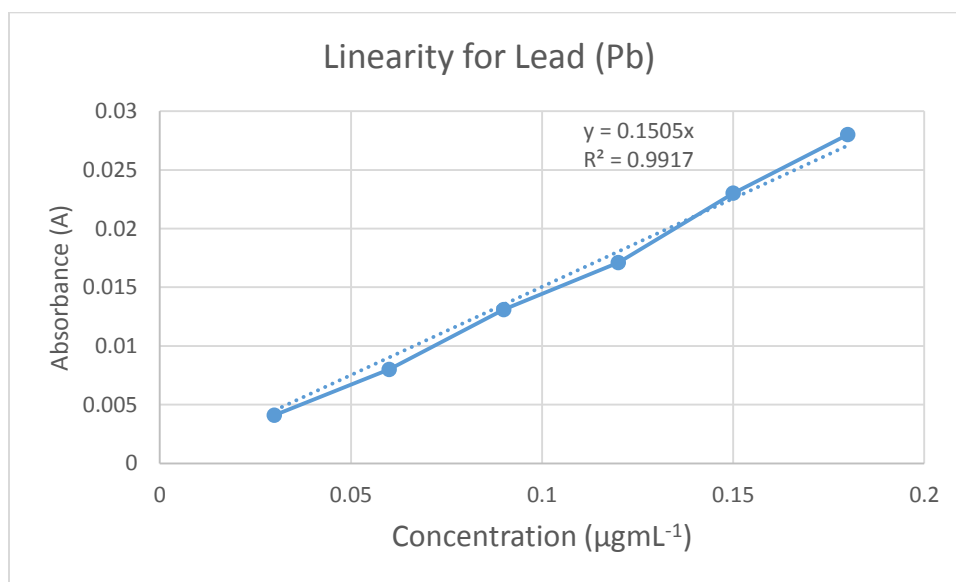


Fig 2. Calibration curve between absorbance and concentration for Lead (Pb)..

Limit of Detection and Quantification

LOD is the minimum analyte concentration detectable but not necessarily quantifiable (Gonzales & Herrador, 2007), whereas LOQ is the lowest concentration quantified with accuracy. To determine the LoD and LoQ for the developed method, ICH guidelines were followed, utilizing standard deviation (σ) and the corresponding slopes (S). The standard deviations were calculated from calibration curve data and using the formulae for LoD and LoQ as $\text{LoD} = 3.3 \times \sigma / S$; $\text{LoQ} = 10 \times \sigma / S$. (S = slope obtained from the calibration curve and σ = standard deviation). LoD of Cd and Pb were estimated as 0.052 and 0.16 $\mu\text{g/mL}$, whereas LoQ were 0.159 and 0.51 $\mu\text{g/mL}$, respectively (Table 03)..

Precision

Precision is typically measured as the relative standard deviation (RSD) of a data set. The spiking method was used to assess repeatability (intra-day) and reproducibility (inter-day) of the proposed method. Ten blank sample solutions, fortified with 0.2 $\mu\text{g/mL}$ Cd and 1.0 $\mu\text{g/mL}$ Pb standards, were analyzed under similar conditions. The % RSD values obtained (Table 05) were less than 3 and were in the acceptable range.

Table 5. Precision Inter-day and intra-day.

	Intra-day			Inter-day		
	Results*	%RSD	%Recovery	Results*	%RSD	%Recovery
Cadmium	9.11	1.89	101.32	9.03	2.45	110.42
Lead	39.5	1.43	99.76	39.89	2.16	98.62

*(Mean of 03 replicates and in percentage)

Accuracy

Accuracy refers to the closeness of the test results to the true value. The samples (orange juice) were spiked with 0.2µg/mL, 0.4µg/mL, and 0.6µg/mL of Cd and Pb working standard concentrations. The result obtained (table 06) ranged between 90.5 to 108.3% which falls under the limits of percent recovery of 80-110%.

Table 6. Accuracy of the proposed method on % recovery of Cd and Pb.

Analyte	Spiking level (µg/mL)	Instrumental result*	% Recovery
Cadmium	0.2	0.20	100.0
	0.4	0.36	92.0
	0.6	0.65	108.3
Lead	0.2	0.18	90.5
	0.4	0.41	102.5
	0.6	0.60	100.0

* (Absorbance)

Sample Analysis

A Proficiency Testing sample provided by (FAPAS) was used as an unknown sample to check the authenticity of the suggested approach. The sample was prepared and aspirated as per the above method and the Cd and Pb content were determined (table 07). The results showed a Z-score of 0.7 and 0.5 for Cd and Pb respectively (Z- score <2) showing satisfactory results.

Table 7. Analysis of sample.

Metal (µg/L)	Assign Value	Z-Score	Found Value	Z-Score Achieved
Cadmium (Cd)	63.9	< 2	73.63 ± 1.21	0.7
Lead (Pb)	43.6	< 2	48.0 ± 1.03	0.5

A Comparison of methods for quantitative measurement of lead (Pb) and cadmium (Cd) in juices is cited in (table 08). It is evident that the present method has low LOD and LOQ and is cost-effective while maintaining accuracy.

Table 8. Comparison of Methods for Cd and Pb Detection in Citrus Juice.

Technique	Sample	LOD (Cd/Pb) $\mu\text{g/mL}$	LOQ (Cd/Pb) $\mu\text{g/mL}$	Accuracy (Cd/Pb) %	Instrument involved and Running cost	Reference
*ICP-OES	Mango, Orange, peach, Guava, Pineapple, grapes Juices	0.0000013 / 0.0000233	0.000002/ 0.00003	NM	High	(Mohammad <i>et. al.</i> , 2020)
*ICP-MS	Canned Citrus Juices	0.0005 / 0.0033	.0017 / 0.0111	103/ 100	High	(De Souza <i>et. al.</i> ,2022
*GFAAS	Soft drinks orange, apple, and others	0.147 / 0.43	0.44 / 1.29	100.4/ 99.6	Moderate	(Alkhatib <i>et. al.</i> , 2020)
GFAAS	Orange juice	0.052/0.16	0.159/ 0.51	100/97.3	Low	This Article

*: Microwave assisted digestion; NM: Not Mentioned

CONCLUSION

Orange juice is a complex matrix containing sugars, acids, and pulp, which may interfere with Cd and Pb detection, the slow and high-temperature digestion along with the application of instrument's background correction is necessary for accurate Cd and Pb detection in the juice sample. This study developed, verified, and presented a novel spectroscopy-based method for the quantitative measurement of lead (Pb) and cadmium (Cd) in fresh orange juice. The technique effectively quantified the targeted heavy metals and showed outstanding accuracy and precision (with %RSD < 3). The calibration curve's high coefficient of determination (> 0.99) suggests that the procedure is highly linear. To verify the method's dependability, validation entailed assessing crucial factors like accuracy, linearity, precision, and system adaptability. According to the results, this analytical method is effective, quick, accurate, economical, and error-free, which makes it ideal for use in quality control labs.

REFERENCES

- Alkhatib, R. and M. Ataie (2020). Determination of trace lead and cadmium in canned soft drinks in Syria. *Journal of Pharmacy and Bioallied Sciences*, 12(3): 344-350.
- Atasoy, M. (2023). Development of a new sensitive method for lead determination by platinum-coated tungsten-coil hydride generation atomic absorption spectrometry. *ACS omega*, 8(25): 22866-22875.
- Beauchemin, D. (2008). Inductively coupled plasma mass spectrometry. *Analytical chemistry*, 80(12): 4455-4486.
- Butcher, D. J. (2024). Recent advances in graphite furnace atomic absorption spectrometry: a review of fundamentals and applications. *Applied Spectroscopy Reviews*, 59(2): 247-275.
- Bolea-Fernandez, E., R. Clough, A. Fisher, B. Gibson and B. Russell (2024). Atomic spectrometry update: review of advances in the analysis of metals, chemicals and materials. *Journal of Analytical Atomic Spectrometry*, 30: 2617-2693.
- De Souza, M. J., M. C. Barciela-Alonso, M. Aboal-Somoza and P. Bermejo-Barrera (2021). Determination of the trace element contents of fruit juice samples by ICP OES and ICP-MS. *Braz. J. Anal. Chem.*, 9: 49-61.
- Evans, E. H., J. B. Dawson, A. Fisher, S. J. Hill, W. J. Price, C. M. Smith, and J. F. Tyson (2001). Atomic Spectrometry Update. Advances in atomic emission, absorption, and fluorescence spectrometry, and related techniques. *Journal of Analytical Atomic Spectrometry*, 16(6): 672-711.
- Evans, E. H., J. A. Day, C. Palmer, W. J. Price, C. M. Smith and J. F. Tyson (2007). Atomic spectrometry update. Advances in atomic emission, absorption and fluorescence spectrometry, and related techniques. *Journal of Analytical Atomic Spectrometry*, 22(6): 663-696.
- Feo, J. C., M. A. Castro, J. M. Lumbreras, B. De Celis and A. J. Aller (2003). Nickel as a chemical modifier for sensitivity enhancement and fast atomization processes in electrothermal atomic absorption spectrometric determination of cadmium in biological and environmental samples. *Analytical sciences*, 19(12): 1631-1636.

- Fisher, A., P. S. Goodall, M. W. Hinds, S. M. Nelms and D. M. Penny (2003). Atomic spectrometry update. Industrial analysis: metals, chemicals and advanced materials. *Journal of Analytical Atomic Spectrometry*, 18(12): 1497-1528.
- Kadhim, I. M. H. and T. T. Miteb (2024). Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Instrumentation, Analysis, Strengths, Limitations, Biomedical and Pharmaceutical Applications. *Clinical Images and Case Reports*, 2(6): 22-42.
- Liu, H., H. Cui, Y. Wang, Z. Jiang, L. Lei and S. Wei (2023). Accurate determination of trace cadmium in soil samples with graphite furnace atomic absorption spectrometry using metal-organic frameworks as matrix modifiers. *Applied Spectroscopy*, 77(2): 131-139.
- Mohamed, F., D. Guillaume, N. Abdulwali, K. Al-Hadrami and M. A. A. Maqtari (2020). ICP-OES assisted determination of the metal content of some fruit juices from Yemen's market. *Heliyon*, 6(9): 00-00.
- Ortner, H. M., E. Bulska, U. Rohr, G. Schlemmer, S. Weinbruch and B. Welz (2002). Modifiers and coatings in graphite furnace atomic absorption spectrometry—mechanisms of action (a tutorial review). *Spectrochimica Acta Part B: Atomic Spectroscopy*, 57(12): 1835-1853.
- Patriarca, M., N. Barlow, A. Cross, S. Hill, A. Robson, A. Taylor and J. Tyson (2021). Atomic spectrometry update: review of advances in the analysis of clinical and biological materials, foods and beverages. *Journal of Analytical Atomic Spectrometry*, 36(3): 452-511.
- Qiao, H. (1992). *Atomization from solutions and slurries in electrothermal atomic absorption spectrometry*. State University of New York at Albany.
- Schlemmer, G. and B. Welz (1986). Palladium and magnesium nitrates, a more universal modifier for graphite furnace atomic absorption spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 41(11): 1157-1165.
- Senila, M. (2024). Recent advances in the determination of major and trace elements in plants using inductively coupled plasma optical emission spectrometry. *Molecules*, 29(13): 3169.
- Subramanian, K. S. (1996). Determination of metals in biofluids and tissues: sample preparation methods for atomic spectroscopic techniques. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 51(3): 291-319.
- Volynsky, A. B. (2000). Mechanisms of action of platinum group modifiers in electrothermal atomic absorption spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 55(2): 103-150.
- Volynsky, A. B. (2004). Comparative efficacy of platinum group metal modifiers in electrothermal atomic absorption spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 59(12): 1799-1821.
- Welz, B. and M. G. R. Vale (2019). Atomic absorption spectrometry and related techniques. In *Ewing's Analytical Instrumentation Handbook, Fourth Edition* (pp. 71-116). CRC Press.
- Wheal, M. S., T. O. Fowles and L. T. Palmer (2011). A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. *Analytical Methods*, 3(12): 2854-2863.
- Wierzbicka, E., B. Vaghefinazari, M. Mohedano, P. Visser, R. Posner, C. Blawert and R. Arrabal (2022). Chromate-free corrosion protection strategies for magnesium alloys — a review: part II — PEO and anodizing. *Materials*, 15(23): 8515.
- Xu, Y. and Y. Liang (1997). Combined nickel and phosphate modifier for lead determination in water by electrothermal atomic absorption spectrometry. *Journal of Analytical Atomic Spectrometry*, 12(4): 471-474.
- Zong, Y. Y. (1996). *Atomization, determination and distribution of lead in bone by electrothermal atomic absorption spectrometry*. State University of New York at Albany.

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