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Degree in Chemistry

FINAL PROYECT

Development of a method for Cadmium determination in lipstick. Considerations about the digestion process assisted by microwaves and the matrix interferences in the atomic absorption technique

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Abstract

This study aims to determine cadmium (Cd) in frequently used cosmetic products, such us lipsticks. Eighteen samples of lipstick (different colors and brands) and one labial protector were analyzed. Some of them were bought in local stores and some were given from the laboratory staff. The content of Cd was measured by flame-atomic absorption spectrometry (AAS). The analyses were preceded by microwave -assisted acid digestion of the samples. The analytical curve for cadmium was linear in the range of 0.250-2.00 mg/L with a correlation coefficient of 0.9964. The limit of detection and the relative standard deviation were estimated at 0.165 mg/L and 0.26 % respectively. Accuracy was assessed trough a recovery test (97.5%). The calibration standards and samples regression slopes were compared to detect if there was a matrix effect, concluding that no statistically significant differences were observed when applying the *F*-test and *t*-test at 95% confidence level. The proposed method is a simple, cheap and could be used as an alternative method to determine Cadmium in lipstick samples.

Resumen

El propósito de este estudio es la determinación de cadmio en productos cosméticos usados frecuentemente, como los pintalabios. Se analizaron dieciocho muestras de pintalabios (diferentes colores y marcas) y un protector labial (cacao). Algunos de ellos fueron comprados en tiendas locales mientras que otros fueron proporcionados por el personal del laboratorio. El contenido de Cd fue medido usando el espectrofotómetro atómico de absorción con llama (AAS). El análisis fue precedido por una digestión ácida en microondas de las muestras de pintalabios. La curva de calibración presentaba linealidad entre 0.250.-2.00 mg/L con un coeficiente de correlación de 0.9964. El límite de detección y la desviación estándar relativa se estimaron en 0.165 mg/L y 0.26% respectivamente. La exactitud se obtuvo a partir de un test de recuperación (97.5%). Las pendientes de regresión de los patrones de calibración y las muestras fueron comparadas para detectar si había efecto matriz, concluyendo que no había diferencias significativas al aplicar la prueba *F* y *t* al 95% de nivel de confianza. El método propuesto

es sencillo, barato y puede considerarse como un método alternativo a los que se usan habitualmente en la determinación de cadmio en pintalabios.

1. Introduction

1.1. Cadmium occurrence and toxicity

Cadmium was discovered in 1817 by Friedrich Stromeyer. The name is derived from the latin ¨cadmia¨, the name for the mineral calmine. It belongs to the group 12 in the periodic table and to the 5th period. It has an electron configuration of 4d^{10} 5s^{2} and it has a relative atomic mass of 112.412 g/mol.[1] Cadmium is mostly used in industrial processes such us anticorrosive agent in PVC products or as a color pigment[2]. Although products containing Cd are recycled to avoid Cd pollution, the first cause of Cd exposure is due to the dumping and incinerating of Cd waste. Moreover, Cadmium is a severe dangerous contaminant due to the extent that it is distributed in the environment. Cd can cause serious health hazards such as both acute and chronic poisoning, pathological change of organs and diseases related to cardiovascular, kidney, bone and liver causing cancer owing to excessive accumulation in human body.[3]

Basically, there are three possible ways of cadmium resorption: Gastrointestinal, pulmonary and dermal.

- Digestive system:

The daily ingestion is approximately 5%, where most of the Cd is taken up from food and drinks. Smokers have an additional intake of Cd, due to fact that Cd can be absorbed from the smoke of cigarettes. Furthermore, a high fiber diet can increase Cd intake.

- Respiratory system:

As it was mentioned before one way of Cd intoxication is by inhaling smoke from cigarettes. The human lung resorbs 40-60% of Cd from the tobacco smoke. Moreover, people who work in industries are exposed to Cd fumes, that have been reported to develop respiratory distress syndromes. Some of the Cd, that has not been retained reaches blood circulation in a form of Cd-cysteine complexes.[3]

The main organs which are damaged after a long exposure to Cd are:

- Kidney
- Teeth
- Bones (low grade of bone mineralization, high rate of fractures and osteoporosis)
- Sexual organs (production of testosterone and progesterone and It has been proven that Cd can pass the placenta causing uterus fetal death)
- Carcinogenety (renal cancer)[3]

The international Agency research on cancer has categorized Cd in group 2A of carcinogen.[4]

1.2.Cosmetics/lipstick

Personal care products and facial cosmetics are commonly used by millions of consumers daily. These products are directly applied to human skin and mainly produce local exposure to certain ingredients. Albeit, the skin provides a protective barrier, the penetration or use of a substance on the oral cavity, on the face, lips, eyes and mucosa may also produce human systematic exposure. Both natural and synthetic substances may produce local effects in human skin such us irritation, sensitization, allergy and photoreactions.[5]

Lipsticks and eye shadows are a group of cosmetics that are most commonly used worldwide. Studies have reported that cosmetic products contain relatively high concentrations of heavy metals. Lipsticks have many types of components such us: antioxidants, pigments, waxes, oils and inorganic components like silica, TiO_2, copper, aluminum and bronze. Cadmium is considered as an impurity in pigments of lipsticks and can be absorbed by children´s and women´s skin. [4]

One way of obtaining Cd as an impurity is by water used in the production of lipsticks. Most of the producers of cosmetics use tap water from urban zones, which contain inorganic components such us: ammonia, phosphates, arsenic, boron as well as metals like: chromium, zinc, iron, copper, manganese, nickel, beryllium and cadmium.[6]

1.3.International regulation

Furthermore, to control the amount of Cd that lipsticks can contain, the United States of America and the European Union have established regulations to govern the safety of cosmetics products. However, cosmetic products and most of their ingredients are not subject to pre-market approval and product safety is mainly the responsibility of the manufacturer. The final responsibility is deferred to the consumers, who choose to use these products under their own risk. The main goal of the governments is to protect the consumer by insuring safe levels of ingredients in products. Even though, governments are trying to apply safety regulations, most regulatory agencies, do not consider a small amount of impurities as posing a risk to human health. [7]

The United States of America, specify that metal concentration limits depend on each additive and its color. On the one hand, Brazil has banned the use of As, Cd, Cr and Pb in the production of cosmetic products. What´s more, cosmetics that are used by children must be easily and safely removed to avoid the possibility of accidental ingestion. On the other hand, countries like Germany and Canada have different regulations. While Canada allows a maximum concentration of 3 mg/Kg of Cd, Germany permits 5 mg/Kg of Cd.[8]

Nowadays, European Directives has banned the use of Cd and other metallic ions or salts in the preparation of cosmetic formulations.[9]

1.4.Analytical techniques and procedures

Samples treatment is crucial for determination of heavy metals in cosmetics. Most of the procedures achieve complete digestion of cosmetic matrices using mixtures of concentrated acids like: nitric acid, sulfuric acid and perchloric acid with hydrogen peroxide (oxidant) in an open system at high temperature. To accelerate dissolution and digestion of these complex matrices, small amounts of hydrofluoric acid (about 1 mL) are usually added to the tank. Attention to safety is necessary when hydrofluoric acid is used because it is extremely corrosive. A microwave digester and high-pressure vessel are commonly utilized to improve digestion efficiency and specificity.

The quantitative techniques used for determination of heavy metals, mainly include inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled atomic emission spectrometry (ICP-AES), inductively coupled plasma mass

spectrometry (ICP-MS), flame atomic absorption spectrometry (F-AAS) and graphite furnace atomic absorption spectrometry. [10]

Among all of them, ICP-MS is a powerful instrument for trace analysis of metal. However, it is not easy to operate and has a high cost of purchase and high maintenance. On the other hand, FAAS is a standard method commonly used to perform elemental determinations and offers a fast and accurate analysis[11]. Researchers have mostly used a hollow cathode lamp. The biggest difference between the articles was the conditions and the acids applied in the digestion procedure, while most of the procedures used nitric acid, hydrogen peroxide or perchloric acid, there were some of them that used hydrofluoric acid. The use of this acid forces the use of special equipment covered with Fluorine, such as burners and recipients, since the most often used equipment is not resistant to this acid.

1.5.Objectives

Considering all the methods and techniques based on the detection of "metals" in cosmetics, the aims of this work were:

- To review the bibliography of the techniques and procedures used in the determination of metals in cosmetic products.
- To develop and validate a simple and low-cost method that could be a feasible and alternative method to determine Cd in lipsticks.
- To apply to the analysis of lipsticks and other samples and interpret the results.

2. Experimental

2.1.*Reagents and samples*

All the reagents used in this procedure, such as: HNO_3, H_2O_2 and Cadmium (Cd) were of high quality. Furthermore, the solutions were prepared with distilled water and a dilution of nitric acid. All the used reagents and solutions for the determination of Cadmium, are outlined below:

- HNO_3 (65%): Nitric acid 65% pure from PanReac AppliChem
- Cadmium standard solution Cd=1.000 ±0.002 g/L AA from PanReac AppliChem.

- H_2O_2: Hydrogen peroxide 30% extra pure stabilized from Riedel-deHaën.
- Solution of HNO_3 (10%) (500 mL): For the preparation of the solution, 50 mL of concentrated nitric acid was measure in a graduated cylinder and added to a 500-mL graduated cylinder with 450 mL of distilled water. Considering that, this mixture is exothermic, first, 50 mL of distilled water were added, then 50 mL of nitric acid and after that, more distilled water was add in order to reach required volume.
- Solution of Cd 20 mg/L (250 mL): 5 mL of Cd were taken from the Cadmium solution of 1000 mg/L. This volume was transfer to a 250-mL flask and filled up with nitric acid (10%).

Some of the samples were acquired in the local markets and the rest of them were given by the staff of the laboratory. The samples analyzed are the following ones.

- ISDIN Stick protector labial FPS15
- Grape Vaseline (Easyparis)
- Orange Vaseline (Easyparis)
- Strawberry lip balm (Easyparis)
- Orange lip balm (Easyparis)
- Red lipstick (Astor)
- Red lipstick (Markwins)
- Orange lipstick (Petite miss)
- Pink lipstick (Petite miss)
- Purple lipstick (Camaleon)
- Purple lipstick (Yolizul)
- Bronze lipstick (Maybelline)
- Red lipstick (Maybelline)
- Pink lipstick
- Green lipstick
- Blue lipstick
- Violet lipstick
- Brown lipstick (Guerlain)

- Red-brown lipstick (Softline Paris)

2.2. *Instrumentation and equipment*

For the digestion of the samples a *speedwave two* microwave digestion system (Berghof, Eningen, Germany) was used (Figure 1.A).

Figure 1.A. Microwave equipment

The speedwave microwave digestion system has been designed to perform chemical digestion procedures under extreme pressure and temperature conditions. Digestion is understood to mean the decomposition of a solid material by a suitable digestion reagent at increased temperature in a vessel that is permeable with regard to microwave. The digestion solutions are directly heated through the absorption of the microwave radiation by the polar digestion reagent, which contains ionic components. Digestion reagents used include HNO_3 (65%), HCl, HF, H_3PO_4 and H_2SO_4. Moreover, the maximum initial weight for organic compounds depends on the digestion vessel and the sample´s carbon content. The use of organic solvents is prohibited due to the fact that spontaneous combustion of any solvent vapors that escape into the oven chamber cannot be produced. The speedwave has been specially developed for sample preparation for AAS spectroscopy procedures.

This type of system is designed for pressure digestion at temperatures up to 240 °C in continuous use and pressures up to a maximum of 75 bars. Furthermore, the unit is a microwave oven with a stainless-steel housing and plastic door, equipped with a double mechanical lock. An infrared thermometer, that permits the temperature of the vessel contents to be quickly determined and regulated is used. The measurement physically determines the temperature radiation emitted by the vessel contents and the digestion solution. In addition, the sample temperature in each individual vessel is detected directly and in real time. This is possible because the vessels materials, such as TFM-PTFE and quartz, cannot absorb the radiation in the mid-infrared range. Moreover, the infrared radiation emitted from the surface of the pressure vessels is filtered out. This allows the sample temperature to be controlled directly in each vessel (Figure 1.B).

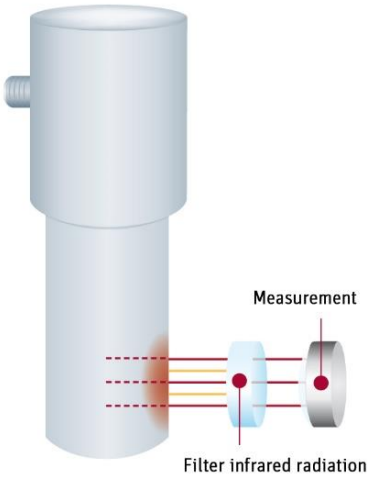

Figure 1.B. Digestion vessel

The advantages of these vessels are[12]:

- Not influenced by microwave radiation.
- All sensors and their circuit lie outside of the microwave field.
- Perfect chemical resistance of sensors in the oven chamber.
- No sensors in the sample vessel. As a result, there is no risk of contamination for the samples and no risk of damage or wear and tear through frequent assembly and disassembly.

The sample analysis was done by atomic absorption spectrometry (AAS). The AAS analysis is based on a liquid sample, which is aspirated into a flame, whose temperature

is 2000-3000K. The liquid evaporates and the remaining solid is atomized (broken into atoms) in the flame. The path-length of the flame is typically 10 cm. Most of the spectrometers use a premix burner, in which fuel, oxidant and sample are mixed before introduction into the flame. The sample solution is drawn into the pneumatic nebulizer by the rapid flow of oxidant, in this case air, past the tip of the sample capillary. Then, the liquid breaks into a fine mist as it leaves the capillary. The spray is directed against a glass bead, upon which the droplets break into smaller particles. The formation of small droplets is named nebulization. A fine suspension of liquid particles in gas, in this case acetylene, is called aerosol. The mist, oxidant and fuel flow past the baffles that promote further mixing and block large droplets of liquid. The aerosol reaching the flame contains only about 5% of the initial sample.[13]

The main working scheme of the atomic absorption is:

Lamp → Flame → Atomic absorption signal→ monochromator→ detector

The source of radiation should present three properties:

- Monochromaticity: only one wavelength
- Intensity: Enough intensity for the wavelength
- Stability: No fluctuations

For measuring Cadmium an electrodeless discharge lamp (EDL) is used. This type of lamp consists of a quartz bulb filled with an inert gas containing the element or a salt of the element for which the lamp is to be used, in this case is Cadmium. The bulb is placed inside a ceramic cylinder on which an antenna for a radio frequency (RF) generator is coiled. When the RF field is applied to the bulb, the inert gas is ionized and the coupled energy excited the vaporized atoms inside the bulb and that is what causes the emission of a characteristic light. EDL´s offers the advantage of lower detection limits. Furthermore, the useful life of an EDL is considerably longer than that of a hollow cathode lamp of the same element.[12] The EDL lamp used and its components are shown in figure 2.A and B[14].

Figure 2.A. EDL used in the work

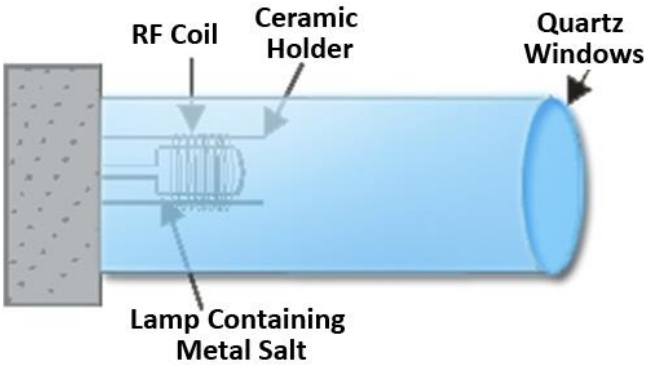

Figure 2.B. EDL components

In this work a AAnalyst 200 Atomic Absorption spectrometer (Perkin Elmer, Shelton, CT, USA) was used with a Cadmium discharge lamp, with a wavelength of 228.80 nm and a 2.7/1.35 nm of slot. The gases used are oxygen and acetylene, with an oxygen flow of 10 L/min and 2.5 L/min for acetylene. In figure 3.A and B it can be seen the atomic spectrophotometer used (AAnalyst 200) and the nebulizer-spray- chamber burner parts.

Figure 3.A. AAnalyst 200 atomic spectrophotometer

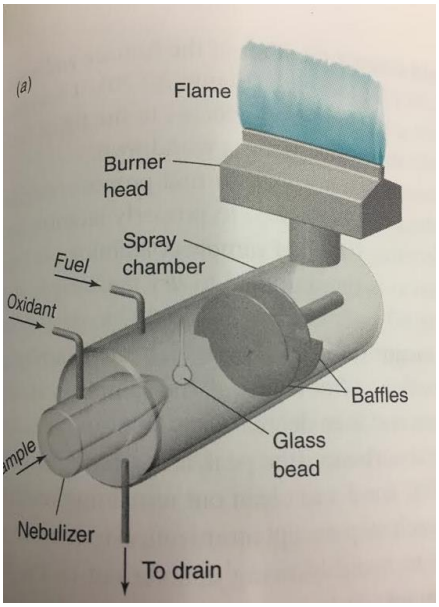

Figure 3.B. Nebulizer-spray chamber components

2.3. Procedure

2.3.1 Sample digestion method

The preparation of the sample involved a digestive method. For the procedure 0.300 grams of the sample was weight and introduced into a digestive vessel. Then, 3 mL of HNO_3 (65%) and 1 mL of H_2O_2 were added. In each assay four vessels were run out. The conditions for the digestion are outlined in table 1.

Table 1. Digestion conditions

Step	1	2	3	4
t_{ramp} (min)	2	5	2	1
t (min)	5	10	10	1
T ºC	135	180	100	75
P factor	400			
I factor	300			
Max power	70%			

t_{ramp}: time needed in order to reach the desired temperature

t: Amount of time, where the temperature is maintained constant

T: temperature

P-I: Algorithm that relates the real temperature and the objective temperature by a relation of the proportional part (P) and the integral (I).

Max Power: Value of power that the microwave can achieve.

After the digestion, the samples were filtered and stored in a 10-mL flask and filled up with HNO_3 (10%). When all the samples were set, they were measure in the atomic absorption spectrometer. The scheme of the procedure is shown in figure 4.

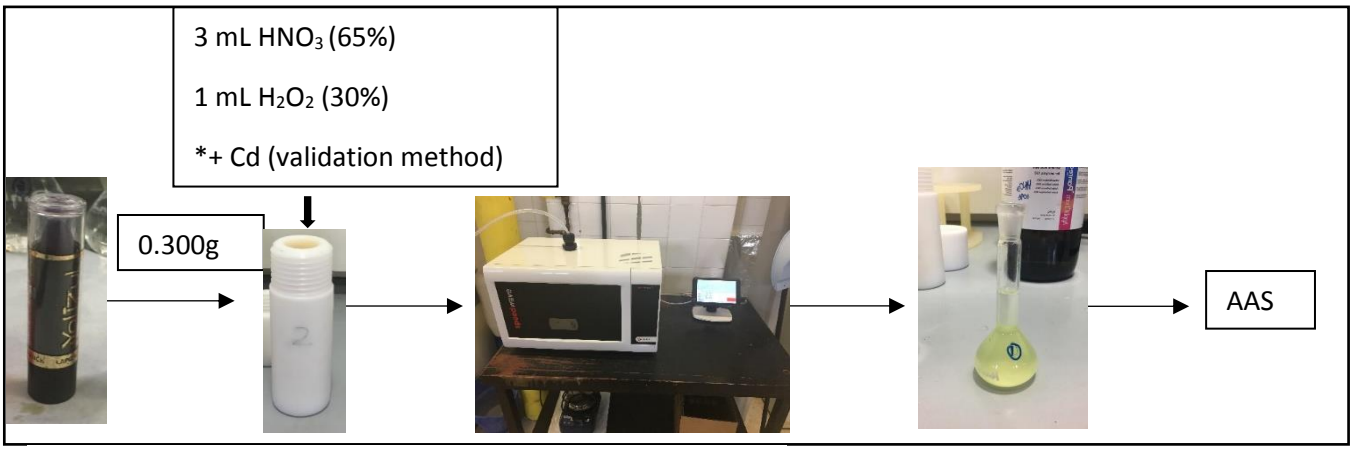

Figure 4. Scheme for the sample digestion method

2.3.2 Atomic absorption determination

For the spectrometric determination of Cadmium, several calibrations with Cadmium were made in order to stablish the best range of concentrations. The selected concentrations were collected in table 2.

Table 2. Standard concentrations for AAS determination of Cd

Standards	1	2	3	4	5	6	7
[Cd] (mg/L)	0.075	0.0125	0.25	0.60	0.75	1.50	2.00

These standards were made from the 20 mg/L Cadmium solution, taking the appropriate volume and filling it up with 10% nitric acid to the 25-mL volume. Once these solutions were prepared, they were measure using the atomic absorption spectrometer with the Cadmium discharge lamp. Before measuring the calibration standards, a water blank was measured to stablish the cero value.

2.4 Validation parameters

The method validation is the process of proving that an analytical method is acceptable for its intended purpose. The parameters that must be evaluated are: specificity, linearity, accuracy, precision, range, recuperation, limits of detection and quantitation and robustness.[15]

- Specificity

It is the ability of an analytical method to distinguish analyte from everything else that might be in the sample.

- Robustness

Is the ability of an analytical method to be unaffected by a small deliberate change in operating parameters.

- Linearity

Measures how well a calibration curve follows a straight line, showing that response is proportional to the quantity of analyte. A common measure of linearity is the square of the correlation coefficient, R^2.

$$R^2 = \frac{[\sum(x_I - \dot{x})(y_i - \dot{y})]\hat{}2}{\sum(x_i - \dot{x})\hat{}2 \sum(y_i - \dot{y})\hat{}2}$$

- Accuracy

It is ¨nearness to the truth¨. Ways to demonstrate accuracy include:

a. Analyze a certified reference material in a matrix like the unknown. The method should find the certified value for analyte in the reference material, within the precision of your method.
b. Compare results from 2 or more different analytical methods. They should agree within their expected precision.
c. Analyze a blank sample spiked with a known addition of analyte. The matrix must be the same as your unknown. When assaying a major component, 3 replicate samples at each of 3 levels ranging from 0.5 to 1.2 times expected sample concentration are customary.
d. If a blank cannot be prepared it is appropriate to make standard additions of the analyte.

   Spiking is the most common method to evaluate accuracy because reference materials are not usually available and a 2nd analytical method may not be readily available. In this case, the accuracy is measure using the spiking method.

- Precision

It is how well replicate measurements agree with one another, usually expressed as a standard deviation. Many types of precision can be distinguished.

a. Instrument precision: ¨Injection precision¨ is the reproducibility observed when the same quantity of one sample is repeatedly introduce (≥10

times) into an instrument. Variability could arise from variation in the injected quantity and variation of instrument response.

b. Intra-assay precision (repeatability): It is evaluated by analyzing aliquots of a homogeneous material several times by one person on one day with the same equipment. Each analysis is independent, so the intra-assay precision is telling us how reproducible the analytical method can be. Intra-assay variability is greater than instrument variability, because more steps are involved.
c. Intermediate precision: Is the variation observed when an assay is performed by different people on different instruments on different days in the same lab. Each analysis might incorporate fresh reagents and different chromatography columns.
d. Interlaboratory precision (reproducibility): Is the most general measure of reproducibility observed when aliquots of the same sample are analyzed by different people in different laboratories.

- Range

Is the concentration interval over which linearity, accuracy and precision are all acceptable.

- Limits of detection and quantitation

The values of these parameters are closely related to the magnitude of the noises in the measurement system. The signal to noise ratio (S/N) is a unidimensional quantity that describes the relationship of an analytical signal to the mean noise levels for a specific sample. The value of this parameter can serve to determine the influence of noise level on the relative measurement deviation. It can be calculated in different ways, but the most common method is the relationship of the arithmetical mean of the results in a measurement series for blind samples to the standard deviation obtained for these series.

a. Limit of detection (LOD): It is the lowest concentration of an analyte that can be detected with statistically significant certainty; this value is n times the noise

level-it is most often 3 times as high. The manner of determining an LOD depends on the following factors:

i. Nature of the analytical method
ii. Characteristics of the applied instrumental technique
iii. Possibilities of obtaining so-called blind samples

If we use a blank for measuring an LOD, the way of obtaining the LOD is by applying the following equation: $LOD = x_0 + 3.3SD$, where x_o is he mean of the blank and SD is the standard deviation. If a blank cannot be used, the values of the regression line can be used instead. The equation for it is: $LOD = bo + 3.3s_{y/x}$, where b_o is the intercept and the $S_{y/x}$ is the value of the residuals.

b. Limit of quantitation (LOQ): It is the quantity or the smallest concentration of a substance that can be determined using a given analytical procedure with an assumed accuracy, precision and uncertainty. This value should be estimated using a suitable standard sample and should not be determined through extrapolation. In order to calculate the LOQ, we multiply the LOD times 3.

$$LOQ = 3xLOD$$

In this work, the values that were measured to validate the method were: Linearity, LOQ, LOD, precision and accuracy (recovery).

3. Results and discussion

3.1 Selection of the microwave conditions

Before the starting of the analysis and the digestion procedure, a literature revision was done. The different conditions used in several works can be seen in table 3.

Table 3. Digestion procedure conditions

Matrix	Quantities	Digestion	Conditions	References
Lipstick	250 mg	H_2O_2 (30%v,v) 2mL HNO_3 (conc) 5mL Triton 1 mL	100ºC 180 min	8
Lipstick	1000 mg	HNO_3 5 mL (conc) H_2O_2 1 mL	80ºC until dried	4
Lipstick	200 mg	HNO_3 4 mL conc H_2O_2 1mL	4 hours 70ºC	7
Lipstick	500 mg	HNO_3 2mL conc	130ºC 15 hours	16
Eye shadow	1000 mg	HNO_3 5 mL 67% HF 1 mL 40%	Irradiated Microwaves Ventilation 3 min	5
Cosmetic matrices	200 mg	Spiking solution 1mL HNO_3 7 mL HF 3 mL	200ºC (MW 1200W) 20 min	11
Face powders	200 mg 200 mg 150 mg	HF 2mL HNO_3 7 mL HNO_3 3 mL H_2O_2 1 mL HNO_3 1 mL HCl 3 mL	20 min 130ºC (MW 1000w) 30 min 200ºC (MW 1000w) Cooling 20 ml H_3BO_3 MW 25 min 1000w MW 400-600w MW 500w Digested 3 times	9
Lipstick	250 mg	HNO_3 5 mL 65% HF 2mL 40%	MW 80 w 5 min 170 ºC 40Bar	12

After considering all the different conditions and acids used, it was chosen to use the mixture of 5 mL of HNO_3 (conc) and 1 mL of H_2O_2. Also, the amount of the sample was fixed in 0.3000 g.

3.2 Calibration

The standard solutions were prepared, starting from a 50 or 20 mg/L stock solution of Cadmium (Cd). The first considered range of concentrations for the standards were: 0.25 mg/L, 0.50 mg/L, 1.00 mg/L, 2.00 mg/L, 3.00 mg/L, 4.00 mg/L, 5.00 mg/L and 8.00 mg/L. Therefore, the selection of the concentration was based on the amount of Cadmium that had to be taken in order to prepare the solutions.

Considering the obtained results, the selected concentration was the 20 mg/L of Cd because the volumes that had to be taken were higher than the volumes for the other concentration. After that, these concentrations were prepared and measured in the atomic absorption spectrometer. The obtained results are shown in table 4:

Table 4. Absorption results

Standards [Cd] (mg/L)	Signal
0.25	0.152
0.50	0.185
1.00	0.322
2.00	0.621
3.00	0.876
4.00	1.112
5.00	1.303
8.00	1.701

At a glance, the results for the higher concentrations were very high. Therefore, a new range was prepared. The new concentrations were: 0.125 mg/L, 0.25 mg/L, 0.6 mg/L, 0.75 mg/L, 1.5 mg/L, 2.00 mg/L. These new concentrations were prepared in an aqueous media and their signal was measure. The obtained results are represented in figure 5.

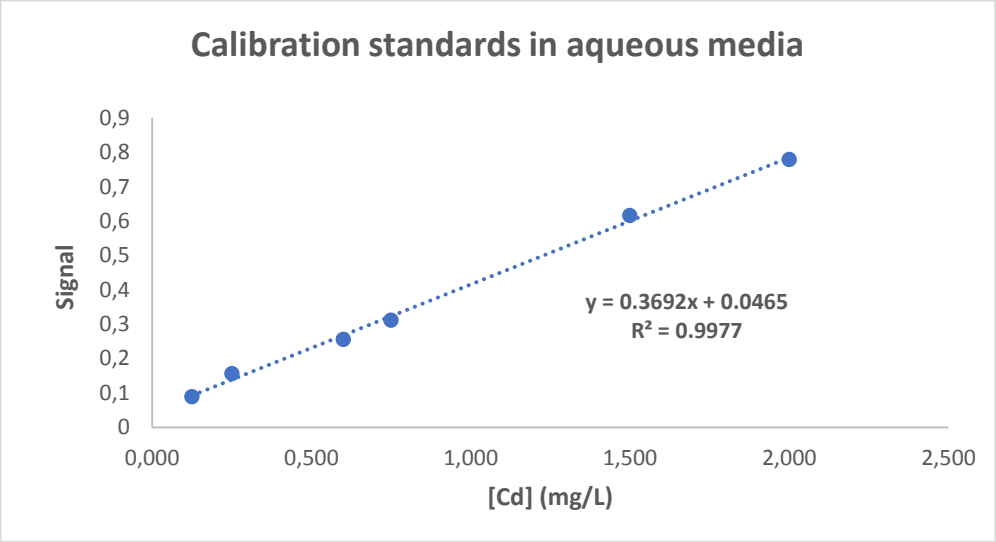

Figure 5. Calibration standards in an aqueous matrix

Knowing that these standards were made in an aqueous solution, it was also considered to do it an acid media, just to see if there was a significant variation in the absorption signal. The solutions were prepared using nitric acid (10% v/v). The obtained results are represented in figure 6

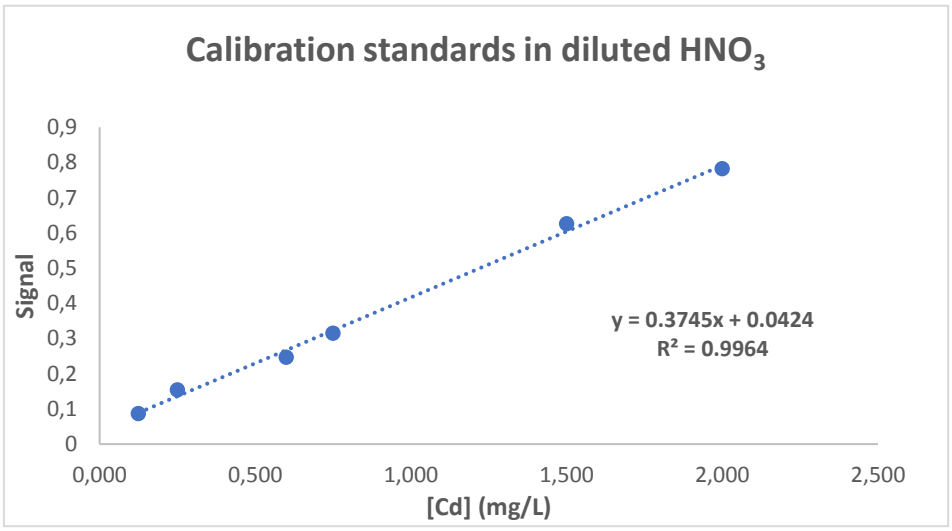

Figure 6 Calibration standards in HNO_3 matrix

Taking into account the obtained results in figures 5 and 6, it can be concluded that the results are quite similar and that there is not a remarkable difference between them. Although, the aqueous standards could be used for further studies, the following

standards were made using nitric acid, considering the resulting standard dilutions after the digestion procedure.

3.3 Matrix effect

Once, the calibration curve was done, the following step was to analyze a reference matrix for lipsticks. The lipstick selected was the Isdin Labial Protector. The aim of this part of the work was to detect if there is a matrix effect.

Matrix is referred to the components of a sample other than the analyte of interest, which in this case is Cadmium. The matrix can have a considerable effect on the way the analysis is conducted and the quality of the results obtained, such effects are called matrix effects. The best approach for accounting for matrix effects is by building a calibration curve using standard samples with known analyte concentration and which try to approximate the matrix of the sample as much as possible. Due to the fact that lipstick samples have complex matrices, the standard addition method is used.[7] For the standard addition method, small known concentrations of the analyte to be determined have been added to aliquots of the unknown sample. These spiked samples as well as the unknown are measured.

The matrix effect, in this case could arise from the Isdin Labial Protector composition. The matrix of this labial stick is quite complex, formed by[17]:

- Ricinus communis (castor) seed oil
- Ozokerite
- Paraffinum liquidum (mineral oil)
- Petrolatum
- Ethylhexyl methoxycinnamate
- Paraffin
- Copernifia cerifera (carnauba wax) wax
- Octyl dodecanol
- C12-15 alkyl benzoate
- Cetyl acetate

- Butyl methoxydibenzoylmethane
- Isostearyl neopentanoate
- Glycol montanate
- Rosa canina fruit oil
- Shorea robusta resin
- Parfum (fragrance)
- Acetylated lanolin alcohol
- Lanolin alcohol
- Tocopheryl acetate
- Panthenol
- Retinyl palmitate
- BHT
- Tocopherol
- CI 77492 (iron oxide)
- CI 15850 (red 6 lake)

In this procedure, first, a 20 mg/L Cd solution was prepared. Then, the standard solutions were made, and this time another lower concentration was included (0.075-2.00 mg/L). Once the standards are all set, the digestion procedure was going to take place, following the steps outlined in the experimental section. After the digestion, two of the four 25-mL flask one with Cadmium and the other one without, were selected in order to carry out the standard addition method. Later on, the standards and the addition standards were measure in the atomic absorption spectrometer. The results obtained for the addition standard method were the collected in table 5.

Table 5. Addition standard results

			Addition Standard method				
Standards	**[Cd] (mg/L)**	**Signal**	**Sample 3**	**Vol of sample (mL)**	**Addition**	**Signal**	**[Cd] (mg/L)**
P1	0.075	0.036	1	3	3 mL water	0.072	0
P2	0.125	0.084	2	3	3 mL P1	0.086	0.0375
P3	0.25	0.150	3	3	3 mL P3	0.167	0.125
P4	0.60	0.252	4	3	3 mL P5	0.227	0.375
P5	0.75	0.297	5	3	3 mL P7	0.466	1.00
P6	1.50	0.619	**Sample 4**	**Vol of sample (mL)**	**Addition**	**Signal**	**[Cd] (mg/L)**
P7	2.00	0.771	1	3	3 mL water	0.074	0
			2	3	3 mL P2	0.115	0.0675
			3	3	3 mL P4	0.180	0.300
			4	3	3 mL P6	0.356	0.75
			5	3	3 mL P7	0.436	1.00

In figure 7, the calibration curve and the sample curve (with Cd) were in the same graph. If the slopes are parallel to each other, that means that there is no matrix effect, but if the sample curve is not parallel to the calibration one; that means that there is a matrix effect.

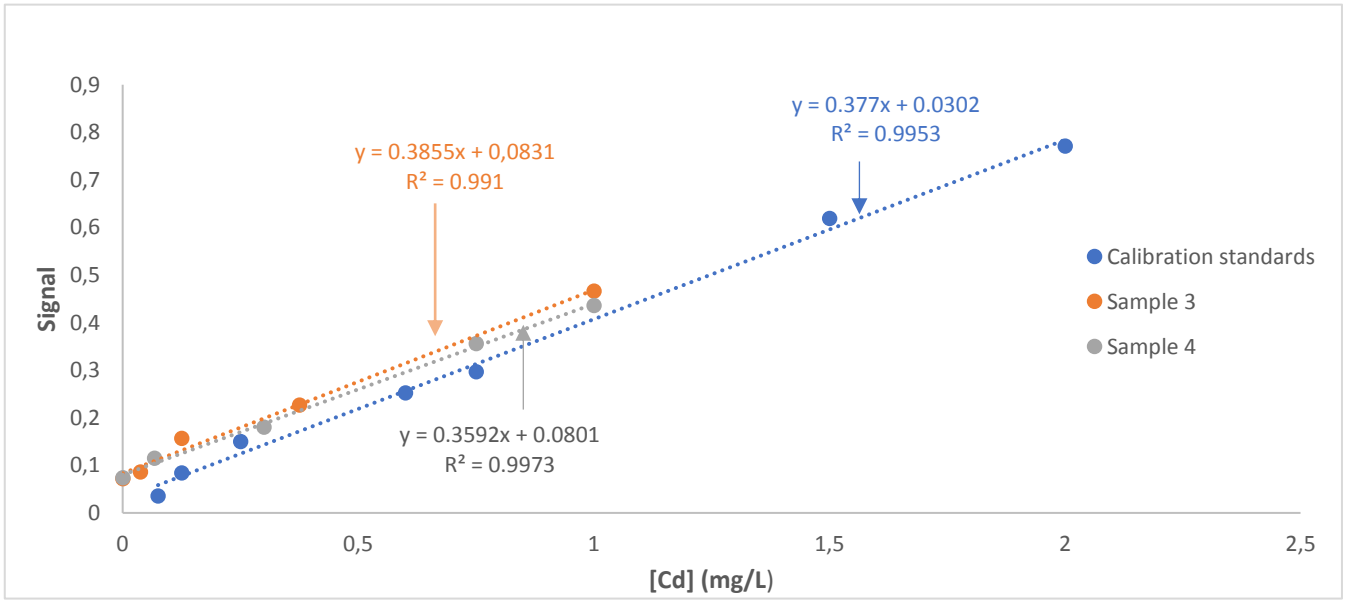

Figure 7. Representation of external calibration standards and addition samples

As it can be seen, the addition sample curves are almost parallel to the calibration curve. So, apparently, there is no matrix effect. Thus, to assure numerically, that there is not a matrix effect of the sample, statistical tests are performed, in order to compare the slopes of the two regression lines. With the obtained plot, the least-squares regression line is obtained and the amount of the analyte present in the sample, x_s, is estimated by extrapolating the line to the abscissa (y=0). In the absence of absolute systematic errors the negative intercept on the concentration axis corresponds to $-x_s$, which consequently $x_s = b_0/b_1$, where b_0 is the intercept and b_1 is the slope. For the homoscedastic situation the standard error of the predicted concentration, which depends on the reliability of b_0/b_1 can be approximated by:

$$S_{x_s} = \frac{S_e}{b_1}\sqrt{\frac{1}{n} + \frac{\bar{y}^2}{b_1^2 \sum(x_i - \bar{x})\text{^}2}} \qquad \textbf{Eq (1)}$$

The comparison of the slopes of the two regression lines (represented as b_{11} and b_{12} respectively) can be performed by means of a *t*-test:

$$t = \frac{b_{11} - b_{12}}{\sqrt{S_{b_{11}}^2 + S_{b_{12}}^2}} \qquad \textbf{Eq (2)}$$

The values for S^2_{b11} and S^2_{b12} can be calculated from:

$$S_{b_{11}}^2 = \frac{S_{e1}^2}{\sum_{i=l}^{n_1}(x_{il} - \overline{x_1})\text{^}2} \quad S_{b_{12}}^2 = \frac{S_{e2}^2}{\sum_{i=l}^{n2}(x_{i2} - \bar{x}_2)\text{^}2} \qquad \textbf{Eq (3)}$$

With n_1 and n_2 the total number of data points in each regression line. If the residuals variances, σ_1^2 and σ_2^2, estimated by S_{ei}^2 and S_{e2}^2 are equal, then the comparison can be performed by means of an *F*-test and the pooled estimated variance is calculated as:

$$S_{ep}^2 = \frac{(n_1 - 2)S_{e1}^2 + (n_2 - 2)S_{e2}^2}{n_1 + n_2 - 4} \qquad \textbf{Eq (4)}$$

The test is then performed by calculating:

$$t = \frac{b_{11} - b_{12}}{\sqrt{S_{ep}^2\left(\frac{1}{\sum(x_{il} - \bar{x}_1)^2} + \frac{1}{\sum(x_{i2} - \bar{x}_2)^2}\right)}} \qquad \textbf{Eq (5)}$$

The test-*t* result should be compared with the tabulated t-value with n_1+n_2-4 degrees of freedom at the chosen significance level (95%).[18]The data of the calibration standards and the samples are collected in table 6.

Table 6. Calibration and samples data

Calibration						
[Standard] (mg/L)	**Signal**	**(x-$\bar{x}$)**	$(x-\bar{x})^2$	$\hat{y}$	$(y-\hat{y})$	$(y-\hat{y})^2$
0.075	0.036	-6.82E-01	4.65E-01	5.85E-02	-2.25E-02	5.05E-04
0.125	0.084	-6.32E-01	4.00E-01	7.73E-02	6.68E-03	4.46E-05
0.250	0.15	-5.07E-01	2.57E-01	1.24E-01	2.56E-02	6.53E-04
0.600	0.252	-1.57E-01	2.47E-02	2.56E-01	-4.40E-03	1.94E-05
0.750	0.297	-7.14E-03	4.90E-05	3.13E-01	-1.60E-02	2.54E-04
1.50	0.619	7.43E-01	5.52E-01	5.96E-01	2.33E-02	5.43E-04
2.00	0.771	1.24E+00	1.54E+00	7.84E-01	-1.32E-02	1.74E-04
$\bar{x}$	$\bar{y}$	**n**	$\hat{y}$	$\sum(x-\bar{x})^2$	$\sum(y-\hat{y})^2$	
0.7571	0.3156	7	0.377x+0.0302	3.243	2.19E-03	
Sample 3						
[Standard](mg/L)	**Signal**	**(x-$\bar{x}$)**	$(x-\bar{x})^2$	$\hat{y}$	$(y-\hat{y})$	$(y-\hat{y})^2$
0	0.072	-0.3075	9.46E-02	8.31E-02	-1.11E-02	1.23E-04
0.0375	0.086	-0.2700	7.29E-02	9.75E-02	-1.15E-02	1.32E-04
0.125	0.157	-0.1825	3.33E-02	1.31E-01	2.57E-02	6.60E-04
0.375	0.227	0.0675	4.56E-03	2.28E-01	-6.00E-04	3.60E-07
1.00	0.466	0.6925	4.80E-01	4.69E-01	-2.60E-03	6.76E-06
$\bar{x}$	$\bar{y}$	n	$\hat{y}$	$\sum(x-\bar{x})^2$	$\sum(y-\hat{y})^2$	
0.3075	0.2016	5	0.3855x+0.0831	6.85E-01	9.23E-04	
Sample 4						
[Standard](mg/L)	**Signal**	**(x-$\bar{x}$)**	$(x-\bar{x})^2$	$\hat{y}$	$(y-\hat{y})$	$(y-\hat{y})^2$
0	0.074	-0.4225	1.79E-01	8.01E-02	-6.10E-03	3.72E-05
0.0625	0.115	-0.3600	1.30E-01	1.03E-01	1.25E-02	1.55E-04
0.300	0.180	-0.1225	1.50E-02	1.88E-01	-7.86E-03	6.18E-05
0.750	0.356	0.3275	1.07E-01	3.50E-01	6.50E-03	4.23E-05
1.00	0.438	0.5775	3.34E-01	4.39E-01	-1.30E-03	1.69E-06
$\bar{x}$	$\bar{y}$	n	$\hat{y}$	$\sum(x-\bar{x})^2$	$\sum(y-\hat{y})^2$	
0.4225	0.2322	5	0.3592x+0.0801	7.64E-01	2.98E-04	

S_{e1}^2 was calculated by applying the following equation:

$$S_{e1}^2 = \frac{\sum(y_1 - \hat{y})\text{^}2}{(n-2)} \qquad \textbf{Eq (6)}$$

The value of S_{e1}^2 for the calibration is 4.39E-04. The next step was to calculate the same value but in this case for sample 3 and 4. The value of S_{e2}^2 that it is referred to sample 3 is 3.08E-4 and the value of S_{e3}^2 referred to sample 4 is 9.93E-05. With these three values, the *F*-value can be calculated by applying the equation:

$$F = \frac{{S_1}^2}{{S_2}^2} \qquad \textbf{Eq (7)}$$

In order to calculate the *F*-value, the slopes of the calibration and the standards have to be used. S_1 will be the one with the highest slope value, while S_2 will be the one with the lowest slope value. In the case of sample 3 and the calibration, S_1 is going to be the value of S_{e1}^2 and S_2 is going to be the value of sample 3.

$$F = \frac{4.39E-04}{3.08E-04} = 1.426$$

The value obtained must be compare with the F_{tab}. The value of this *F* can be obtained taking into account that the confidence interval is 95%. The value is 4.534. So, it can be concluded that:

$$F_{cal} < F_{tab(0.05.4,6)}$$

As judge from the *F*-test, the residual variances can be considered to be similar since F_{cal}=1.426 is smaller than $F_{tab(0.05.4,6)}$=4.534. Consequently, the pooled estimated variance is calculated using equation 4.

$$S_{ep}^2 = \frac{(7-2)*4.39E-04 + (5-2)3.08E-04}{7+5-4} = 3.898E-04$$

Therefore, the *t*-value can be calculated using equation 5:

$$t = \frac{0.377 - 0.3855}{\sqrt{3.89E-04 * (\frac{1}{3.243} + \frac{1}{6.85E-01})}} = 0.324$$

As the calculated t=0.324 is lower than the tabulated $t_{0.05,8}$=2.31, it should be concluded that the slopes of the calibration line and the addition standard line are not significantly different ad that indicates that there is not a matrix effect.

In the case of sample 4 and the calibration S_1 is going to be the value of S_{e1}^2 and S_2 is going to be the value of sample 4.

$$F = \frac{4.39E-04}{9.93E-05} = 4.42$$

The value obtained must be compare with the F_{tab}. The value of this F can be obtained taking into account that the confidence interval is 95%. The value of this F is 4.534. So, it can be concluded that:

$$F_{cal} < F_{tab(0.05.4.6)}$$

As judge from the F-test, the residual variances can be considered to be similar since F_{cal}=4.42 is smaller than $F_{tab(0.05.4,6)}$=4.534. Consequently, the pooled estimated variance is calculated using equation 4.

$$S_{ep}^2 = \frac{(7-2)*4.39E-04+(5-2)9.93E-05}{7+5-4} = 3.116E-04$$

Therefore, the t-value can be calculated using equation 5:

$$t = \frac{0.377-0.3592}{\sqrt{3.116E-04*(\frac{1}{3.243}+\frac{1}{7.64E-01})}} = 0.793$$

As the calculated t=0.793 is lower than the tabulated $t_{0.05,8}$=2.31, it should be concluded that the slopes of the calibration line and the addition standard line are not significantly different ad that indicates that there is not a matrix effect.

During the procedure, some of the samples were store in the fridge until the next week to measure all the samples at the same time. Although, it was not expected a change in the signal value, there was a significant change (50%). The signal value decreased its

value to the half of it. So, all the standards and the additions had to me measure the same day.

3.4 Validation parameters

Once the calibration line was obtained and the matrix effect was checked, now the method had to be validated. In order to validate the method, precision, accuracy, limits of detection and quantitation (LOD and LOQ) and recovery were determined.

Governments and health agencies stablish that the maximum amount of Cd in cosmetic products should be around 3-5 mg/Kg.

In table 7 it can be observed the values that have to be obtained according to the AOAC in the precision and accuracy for the validation of the method[19]:

Table 7. Expected precision (repeatability) and recovery as a function of analyte

Analyte, %	Mass fraction (C)	Unit	RSD %	Mean recovery %
100	1	100%	1.3	98-102
10	10^{-1}	10%	1.9	98-102
1	10^{-2}	1%	2.7	97-103
0,1	10^{-3}	0.1%	3.7	95-105
0,01	10^{-4}	100 ppm (mg/Kg)	5.3	90-107
0,001	10^{-5}	10 ppm (mg/Kg)	7.3	80-110
0,0001	10^{-6}	1 ppm (mg/Kg)	11	80-110
0,00001	10^{-7}	100 ppb (µg/Kg)	15	80-110
0,000001	10^{-8}	10 ppb (µg/Kg)	21	60-115
0,0000001	10^{-9}	1 ppb (µg/Kg)	30	40-120

Regarding the limit of detection (LOD), to validate the result, it must fulfill two conditions[20]:

$$LOD * 10 > c_{min}$$

$$LOD < c_{min}$$

3.4.1. Limits of detection and quantification (LOD and LOQ)

Taking into account the data obtained in the calibration standards, the calculus of the LOD and LOQ were obtained from the deviations of the residuals.

Eq (8)

$$LOD = \frac{3.3 * S_{\frac{y}{x}}}{b}$$

$$LOQ = 3 * LOD$$ **Eq (9)**

We have taken into account six calibration lines obtained during the work. The calibration standards and their signals are shown in table 8.

Table 8. Cd standards and atomic absorption signals

[Standards] (mg/L)	Signal	x^2	$(x-x_m)$ ^2
0.075	0.033	5.63E-03	0.4653
0.075	0.036	5.63E-03	0.4653
0.075	0.036	5.63E-03	0.4653
0.075	0.039	5.63E-03	0.4653
0.075	0.038	5.63E-03	0.4653
0.075	0.037	5.63E-03	0.4653
0.125	0.070	1.56E-02	0.3996
0.125	0.071	1.56E-02	0.3996
0.125	0.071	1.56E-02	0.3996
0.125	0.069	1.56E-02	0.3996
0.125	0.072	1.56E-02	0.3996
0.125	0.072	1.56E-02	0.3996
0.250	0.125	6.25E-02	0.2572
0.250	0.125	6.25E-02	0.2572
0.250	0.128	6.25E-02	0.2572
0.250	0.130	6.25E-02	0.2572
0.250	0.126	6.25E-02	0.2572
0.250	0.124	6.25E-02	0.2572
0.60	0.245	3.60E-01	0.0247
0.60	0.242	3.60E-01	0.0247
0.60	0.244	3.60E-01	0.0247
0.60	0.241	3.60E-01	0.0247
0.60	0.245	3.60E-01	0.0247
0.60	0.245	3.60E-01	0.0247
0.75	0.340	5.63E-01	5.10E-05
0.75	0.339	5.63E-01	5.10E-05
0.75	0.341	5.63E-01	5.10E-05
0.75	0.345	5.63E-01	5.10E-05
0.75	0.342	5.63E-01	5.10E-05
0.75	0.345	5.63E-01	5.10E-05
1.50	0.616	2.25	0.5518
1.50	0.615	2.25	0.5518
1.50	0.614	2.25	0.5518
1.50	0.615	2.25	0.5518
1.50	0.614	2.25	0.5518
1.50	0.615	2.25	0.5518
2.00	0.760	4.00	1.5447
2.00	0.761	4.00	1.5447
2.00	0.762	4.00	1.5447
2.00	0.763	4.00	1.5447
2.00	0.763	4.00	1.5447
2.00	0.759	4.00	1.5447

The average calibration line obtained with data of table 8 is represented in figure 8.

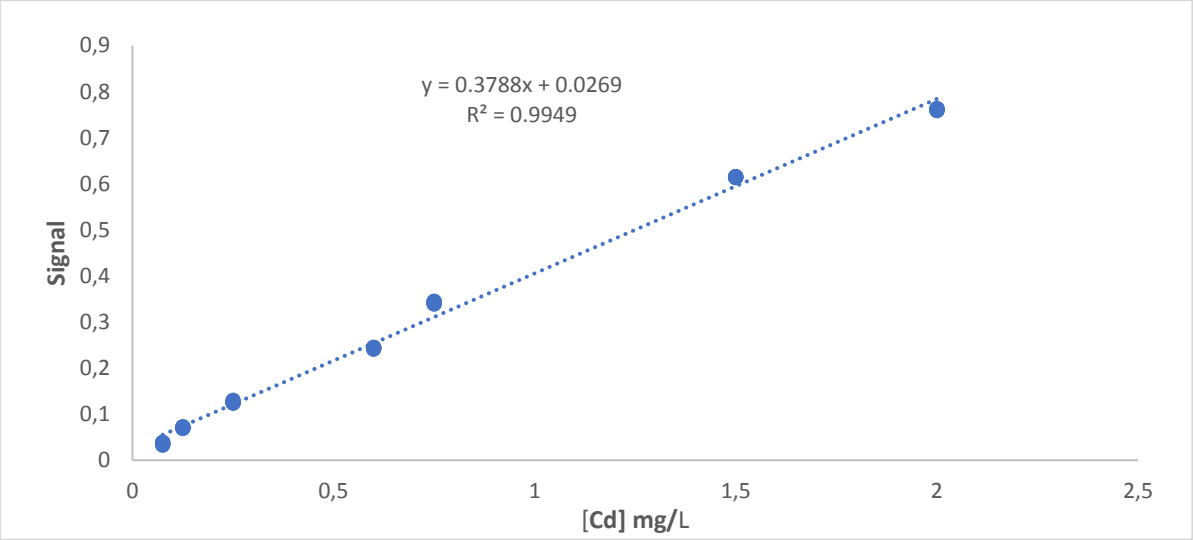

Figure 8. Average calibration line obtained with Cd standards

Table 9 compiles the regression values obtained from table 8.

Table 9. Regression values

X_m	0.7571	**LOD= (3.3*SDxy)/b**	0.165 mg/L
n	42	**LOQ=3*LOD**	0.494 mg/L
Residual SD (SDxy)	0.0189000		
SDb	0.0042844		
SDa	0.0066632		
Reg.coef. -r	0.9974		
R^2	0.9948		

In this case not all the standards concentrations are beyond the LOD (0.165 mg/L). There are two standard concentrations (0.075 mg/L and 0.125 mg/L) that are below the LOD and cannot be considered for the calibration line. If we eliminate those values, the calibration line obtained is acceptable (LOD = 0.165 mg Cd/L / c_{min}=0.25 mg/L) because they fulfill the conditions outlined before and it be concluded that the calibration line is valid for this method. Considering that in analysis of samples 0.3 g were weighted and taken to a 10-mL flask, the value for LOD expressed in the most used units of concentration was 5.5 mg/Kg, which is slightly higher than the value stablished by the regulation. If we compare the result with the values obtained in the literature, most of

the concentration in the samples were below the LOQ. There was just only one case, a pink lipstick from China, which concentration (0.022 mg/Kg) was slightly over the LOQ (0.02mg/Kg).[8] The articles use the residuals to calculate the LOD and LOQ.

3.4.2 Precision

In order to carry out the precision, in a period of a month, 4 days were selected randomly belonging each day to each week. The concentration of Cd used was a 2 mg/L of Cd. The results are shown in table 10.

Table 10. Precision calculus

Test	**Day 1**	**Day 2**	**Day 3**	**Day 4**
1	1.950	1.942	1.955	1.958
2	1.947	1.939	1.947	1.953
3	1.944	1.950	1.944	1.950
4	1.953	1.953	1.95	1.947
x_i (mg/L)	1.949	1.946	1.949	1.952
S_i (mg/L)	3.87E-03	6.58E-03	4.69E-03	4.69E-03
S_i^2 (mg/L)	1.50E-05	4.33E-05	2.20E-05	2.20E-05
RSD (%)	0.198%	0.330%	0.241%	0.241%

$\bar{x}$	1.949 mg/L
S	5.03E-03 mg/L
RSD	**0.26%**

$\bar{x_i}$	1.949 mg/L
$\bar{S_i}$	2.45E-03 mg/L
∑var	1.023E-04 $(mg/L)^2$

The equations used are:

$$RSD = \frac{S}{\bar{x}} * 100 \qquad \textbf{Eq (10)}$$

$$\bar{S}(var) = \sqrt{\frac{\sum var}{4}} \longrightarrow \bar{S}(var)\text{=5.06E-03 mg/L} \qquad \textbf{Eq (11)}$$

As it can be seen in table 10, the relative standard deviation (RSD) in the intermediate precision (0.26%) is lower than the one that is specified in the validation method (table 7A). The value obtained for the repeatability using the variances is 0.26% too. Therefore, it was concluded that the precision of method was valid.

3.4.3 Accuracy

To determine the accuracy of the method, several tests were developed with the ISDIN labial protector, using a 2 mg/L of Cd. Between the several methods to determine accuracy a recovery test was made by performing 4 runs, where Cd was added, in 4 different days. Table 11 shows the results obtained.

Table 11. Recuperation results

Test	**Recovery (mg/L)**				**[Cd]added (mg/L)**
1	1.950	1.942	1.955	1.958	2.00
2	1.947	1.939	1.947	1.953	
3	1.944	1.950	1.944	1.950	
4	1.953	1.953	1.950	1.947	
X_m	1.949	1.946	1.949	1.952	
Recuperation (%)	97.4	97.3	97.5	97.6	

The recovery of Cd in the samples of the ISDIN labial protector is around 97.5%, which means that most of the Cd added has been recuperated. The % of recovery obtained was inside the range of recovery specified in the validation method (7B). Therefore, it can be concluded that the recovery is acceptable and the accuracy of the method is valid.

3.5. Application to samples

After analyzing the referenced matrix, the next step was to apply this method to samples. As it was done before, the first step was to transform the solid samples to a solution using the digestion procedure. In this case, the digestive conditions were the same, but now no Cd is added to the sample. After cooling the samples, they are transfer to a 10 mL-flask. Due to the fact, that the lipstick matrix is more complex, before the signal measure, a filtration must be done. For this, a 0.45 μm filter was used. None of the samples gave a detectable signal value for Cd. Although, none of the samples gave a detectable signal, in other studies the analyzed samples gave a range of concentrations from 0 to 60,2 mg Cd/Kg[21]. Table 12 shows the lipsticks used in the analysis.

Table 12. Lipstick samples results

Lipstick samples	**[Cd] mg/L**
Grape Vaseline (Easyparis)	No detectable
Orange Vaseline (Easyparis)	No detectable
Strawberry lip balm (Easyparis)	No detectable
Orange lip balm (Easyparis)	No detectable
Red lipstick (Astor)	No detectable
Red lipstick (Markwins)	No detectable
Orange lipstick (Petite miss)	No detectable
Pink lipstick (Petite miss)	No detectable
Purple lipstick (Camaleon)	No detectable
Purple lipstick (Yolizul)	No detectable
Bronze lipstick (Maybelline)	No detectable
Red lipstick (Maybelline)	No detectable
Pink lipstick	No detectable
Green lipstick	No detectable
Blue lipstick	No detectable
Violet lipstick	No detectable
Brown lipstick (Guerlain)	No detectable
Red-brown lipstick (Softline Paris)	No detectable

4. Conclusions/Conclusiones

In the development of the atomic absorption spectrophotometric method for determination of Cadmium in lipsticks, assays in acid matrix were performed. After reviewing other research articles of the same topic, it was decided to use a MW digestion with HNO_3 and H_2O_2.

Once the review was done, and the MW digestion conditions were set up; the calibration was carried out obtaining a regression line with a linear range between 0.25 mg/L to 2.00 mg/L, and an R^2=0.9964.

To validate the method, the LOD and LOQ, precision and accuracy were determined. LOD and LOQ values were calculated in acid matrix (HNO_3). The LOD calculated fulfill the

conditions of the bibliographic reference. The values for these to parameters were LOD= 0.165 mg/L (5.5 mg/kg) and LOQ=0.494 mg/L.

To know if the matrix could influence the signal obtained a comparison of the slopes of the calibration line and the samples was carry out. A *F*-test and a *t*-test were performed. The results concluded that there were not significant differences between the slopes with a confidence level of 95%, so the matrix did not have an effect in the signal.

To determine the precision 4 analyses were randomly performed in 4 days. The study was made in the same spectrophotometer, with the same reagents (prepare in the same day), the same analyst, but different days. Repeatability was calculated from the variances and the average deviations, and the intermediate precision with the standard deviation of all the data. The relative standard deviation (RSD) obtained with the intermediate precision was 0.26%, which is lower than the value given in the AOAC validation method.

To determine accuracy, a recovery test was performed by adding analyte (Cd) to samples without Cd (ISDIN Labial protector). The recovery obtained was 97.5%, which meets the acceptable range for the validity of the method specified by the AOAC.

When measuring the samples, none of the lipsticks gave a detectable signal of atomic absorption for Cd. The reason could be due to the fact that the samples do not have Cd, or because the method is not sensitive enough to detect small amounts.

It is an interesting method because it only requires 0.3 g of sample and small volumes of reagents. Instrumentally, it only requires an atomic absorption spectrophotometer and the digestion system.

To sum up, it is concluded that this method is reliable to determine Cd, since the accuracy and precision of this method is obeyed the conditions specified by AOAC. In order to obtain a better LOD, a more sensitive instrumentation such as ICP-MS should be used.

El desarrollo del método para la determinación de cadmio en pintalabios con absorción atómica, se realizó en matriz ácida. Después de revisar otros artículos del mismo tema, se decidió utilizar una digestión ácida de las muestras empleando HNO_3 y H_2O_2.

Una vez establecidas las condiciones de la digestión; se procedió a realizar la calibración, obteniendo una recta de regresión con un rango lineal entre 0.25-2.00 mg/L y con un R^2=0.9964.

Además, para saber si la señal obtenida se veía afecta por la matriz de la muestra, se realizó una comparación de las pendientes de regresión del calibrado y de las muestras. Para ello, se llevó a cabo un test-*F* y un test-*t*, donde se vio que no había diferencias significativas entre ambas pendientes con un nivel de confianza del 95%.

En la validación del método, se determinaron en LOD, LOQ, la precisión y la exactitud. Los valores de LOD y LOQ fueron obtenidos a partir de una matriz ácida (HNO_3). El valor obtenido para el LOD cumplía las condiciones mencionadas en la bibliografía. Los valores para estos parámetros fueron LOD= 0.165 mg/L (5.5 mg/Kg) y LOQ= 0.494 mg/L.

Para la determinación de la precisión, se realizaron 4 análisis en 4 días al azar en el mismo mes. Para ello, se realizó el estudio en el mismo espectrofotómetro, con los mismos reactivos (preparados en el mismo día) y con el mismo analista en diferentes días. Se calculó la repetibilidad a partir de las varianzas y con la media de las desviaciones. La precisión intermedia se calculó con la desviación estándar de todos los datos. La desviación estándar relativa (RSD) obtenida mediante la precisión intermedia fue de 0.26%. Este % obtenido es un valor inferior al dado por la AOAC por lo que la precisión de este método es válida.

Para determinar la exactitud, se realizaron ensayos de recuperación añadiendo analito (Cd) a las muestras que no tienen Cd (Protector Labial ISDIN). La recuperación que se obtuvo tenía un rango de recuperación promedio de 97.5%, el cual cumple un rango aceptable y está dentro del rango estipulado por la AOAC, por tanto, la exactitud del método se da por válida.

La medición de la señal de los pintalabios analizados no dio señales detectables. La razón se puede deber a que ninguno de ellos tenía cadmio, o a que el método no es lo suficiente sensible como para poder determinar concentraciones muy bajas.

Es de reseñar que, es un método interesante puesto que solo se precisa 0.3 g de muestra para llevar a cabo el análisis; referente a la instrumentación, solo se necesita el espectrofotómetro y el microondas para la digestión.

Teniendo en cuenta todo lo mencionado anteriormente, y que tanto la exactitud como la precisión están dentro de los valores estipulados por la AOAC, se puede concluir que es un método válido para determinar Cd en pintalabios, aunque para obtener un mejor LOD, sería aconsejable utilizar un equipo más sensible como puede ser el ICP-MS.

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