ANALYTICAL CHEMISTRY LABORATORY II

CHEMISTRY DEGREE

ACADEMIC YEAR 2019-20

Analytical Chemistry Department
Universitat de València

INDEX

	CHEMISTRY DEGREE **Department of Analytical Chemistry** Subject: **Analytical Chemistry Laboratory II** **PRACTICE 1: DETERMINATION OF CAFFEINE AND PARACETAMOL IN PHARMACEUTICALS BY DERIVATIVE SPECTROSCOPY AND MULTIPLE LINEAR REGRESION**	PRACTICE 1

1.- OBJECTIVE

Simultaneous determination of two compounds by derivative spectroscopy and multiple linear regression.

2.- BASIS

Paracetamol (Figure 1a) is a widely used analgesic and antipyretic because of its few side effects at the digestive level. Caffeine (Figure 1b) is present in many pharmaceutical preparations as it enhances the effect of analgesics and is also a stimulant that reduces the sedative effect of some active ingredients.
As can be seen in figure 2, both substances have overlapping absorption spectra. Therefore, when a direct determination is carried out, both interfere with each other.
Figure 2 shows that caffeine presents, in basic medium, a band whose maximum is around 270 nm, while paracetamol, in basic solution, has a maximum absorption above 250 nm.
To avoid the interference, when two substances have overlapped spectra, it is possible to carry out the determination either individually by using derivative spectrophotometry or simultaneously by a multiple linear regression model.

Figure 1.- Molecules of paracetamol (a) and caffeine (b).

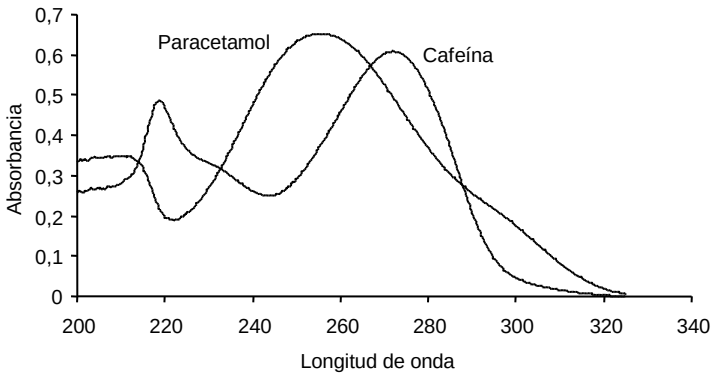


Figure 2.- UV/Vis spectra of caffeine and paracetamol.

Multiple linear regression

Assuming additivity of the absorbance signals and absence of other absorbent compounds, the absorbance of a binary caffeine/paracetamol mixture at any wavelength *i* can be expressed as follows:

$$A_{\lambda_i} = (\varepsilon_P)_{\lambda_i}\, b\, C_P + (\varepsilon_C)_{\lambda_i}\, b\, C_C \qquad (1)$$

Where:

A = absorbance

λ_i = wavelength i

ε_P = absorptivity of paracetamol

ε_C = absorptivity of cafeïne

b = length of the sample cell

C_P =paracetamol concentration

C_C= caffeine concentration

Therefore, at two wavelengths λ1 and λ2, a system of equations can be proposed whose resolution allows the calculation of the content of paracetamol and caffeine in the measurement solution, provided that the molar absorptivities (ε) of both substances at the working wavelengths are known.

$$A_{\lambda_1} = (\varepsilon_P)_{\lambda_1}\, b\, C_P + (\varepsilon_C)_{\lambda_1}\, b\, C_C \qquad (2)$$

$$A_{\lambda_2} = (\varepsilon_P)_{\lambda_2}\, b\, C_P + (\varepsilon_C)_{\lambda_2}\, b\, C_C \qquad (3)$$

To calculate the molar absorptivitiy, it is sufficient to obtain the calibration lines of each substance at the two working wavelengths.

Derivative Spectroscopy

The determination can also be performed by derivative spectroscopy. For this purpose, the binary mixture should be considered to consist of an analyte and an interfering substance, e.g. paracetamol (analyte) and caffeine (interfering substance). If we derive Eq. (1) with respect to the wavelength, the expression is obtained:

$$\frac{dA_{\lambda_i}}{d\lambda_i} = b\, C_P \frac{d(\varepsilon_P)_{\lambda_i}}{d\lambda_i} + b\, C_C \frac{d(\varepsilon_C)_{\lambda_i}}{d\lambda_i} \qquad (4)$$

At wavelengths in which the derivative of the interfering substance (caffeine) is zero, we can pose the

following equation:

$$\frac{dA_{\lambda_i}}{d\lambda_i} = b\, C_P \frac{d(\varepsilon_P)_{\lambda_i}}{d\lambda_i} \qquad (5)$$

This expression allows us to carry out the determination of the substance of interest, in this case, paracetamol.
In the same way, the determination of caffeine can be carried out by considering paracetamol as the interfering substance.

3.- RISK PREVENTION AND WASTE MANAGEMENT

Carefully read the safety data sheets of the reagents used. The waste must be poured into appropriate containers which will be properly labelled in the laboratory.

4.- MATERIAL, REAGENTS, INSTRUMENTATION (*see ANNEX I*)

Reagents: Caffeine standard solution of 100 mg/L, Paracetamol standard solution of 100 mg/L, Carbonate/bicarbonate buffer solution 0.5 M at pH=10.5 (prepared from Na_2CO_3 •$2H_2O$ and concentrated HCl) **(prepared).**

Instrumentation: A dual-beam UV-Vis spectrophotometer with quartz cuvettes with 1 cm optical pathlength.

Sample: pharmaceutical preparation containing as active ingredients caffeine and paracetamol. (Expected concentration: Caffeine 15-25%, Paracetamol 35-50%).

5.- PROCEDURE

5.1.- Preparation of the sample

Weigh approximately 0.1 g of the pre-shredded tablet (in triplicate). Dissolve each of the three replicas in 250 mL beakers with about 50 mL of water and filter (only if necessary) with a filter plate (number 4). Transfer the dissolutions or filtered to 100 mL volumetric flask, add the washing water from thebeaker to the gauged and gauge with distilled water. Then, taking into account the expected content, prepare the measuring solutions by diluting the initial sample solutions and in the presence of a 0,05 M buffer mixture (final volume 25 mL).

5.2.- Determination by multiple linear regression

The procedure requires obtaining the response coefficients of paracetamol and caffeine at the two working wavelengths. To do this, proceed as follows:

From standard solutions of caffeine and paracetamol of 100 mg/L each, a series of standard solutions are prepared in the concentration range 0 to 10 mg/L and in the presence of a 0.05 M buffer mixture (final volume 25 mL).

The absorption spectra are then obtained for each of the calibration solutions and the three sample solutions, the maximum absorption of caffeine and paracetamol are located in the working conditions, the calibration lines are constructed at both wavelengths and once the response coefficients have been calculated, the system of equations is solved that allows the caffeine and paracetamol content to be obtained in the sample solutions.
Finally, once the concentrations of caffeine and paracetamol have been found in each measurement solution, the percentage is found in the original sample.

5.3.- Determination by derived spectroscopy

After localization of the caffeine and paracetamol nullification wavelengths in the first-order derived spectra, the working wavelengths are selected to carry out the caffeine and paracetamol determination.

At the annulment wavelength of paracetamol, the signals derived from the caffeine standard solutions are obtained, the corresponding calibration line is constructed and the concentration of caffeine in the sample is obtained by interpolation.

In the same way, at the caffeine cancellation wavelength, the signals derived from the paracetamol standard solutions are obtained, the corresponding calibration line is constructed and the concentration of paracetamol in the sample is obtained by interpolation.

Finally, once the concentration of caffeine and paracetamol has been found in each measurement solution of the sample, the mean concentration in the original sample is found. Statistically compare the results of both methods for a 95 % confidence level.

6.- REFERENCES

- "Laboratorio de Análisis Instrumental", A.Maurí, M.Llobat and R.Herráez. Servei de Publicacions de la UV and Editorial Reverté (2010).

- "Analytical Chemistry", G. Christian, McGraw-Hill, 6th edition, 2010.
- "Principles of Instrumental Analysis", D. A. Skoog, F.J. Holler, S.R. Crouch. Paraninfo, 6th edition, 2009.

7.- QUESTIONS

1.- In the simultaneous determination of two substances by multiple linear regression, why are two wavelengths sought where the differences in absorbance are large? What would happen in the extreme case that the spectra do not overlap?

2.- Taking into account the caffeine and paracetamol spectra shown in Figure 2, indicate which wavelengths could be of interest for the simultaneous determination of paracetamol and caffeine. Reason the answer.

3.- Indicate which wavelengths have been used to carry out the determination of paracetamol by means of derivative spectroscopy. Reason the answer.

4.- A pharmaceutical preparation only contains 300 mg of paracetamol and 100 mg of caffeine. Indicate how you would treat the sample and which dissolutions and dilutions you would make for the determination of both compounds using the calibration lines obtained in this practice.

1.- OBJECTIVE

Study of variables affecting molecular fluorescence: selection of wavelengths and study of linearity. Determination of quinine in tonic water.

2.- BASIS

A fluorimetric determination can be carried out either after the formation of a fluorescent derivative or by taking advantage of the compound's native fluorescence. In any case, it is necessary to know "a priori" the fluorescent characteristics of the molecule, and the location of the wavelengths of maximum excitation and emission are crucial parameters. For the selection of these wavelengths, it is necessary to obtain the excitation and emission spectra beforehand. Both spectra depend, like the absorption spectra, on the energy levels of the molecule.

If the fluorescent behavior of a molecule is unknown, it is useful to obtain the absorption spectrum beforehand and locate its maximum, since it will theoretically coincide with the maximum of the excitation spectrum, although in practice slight differences may occur.

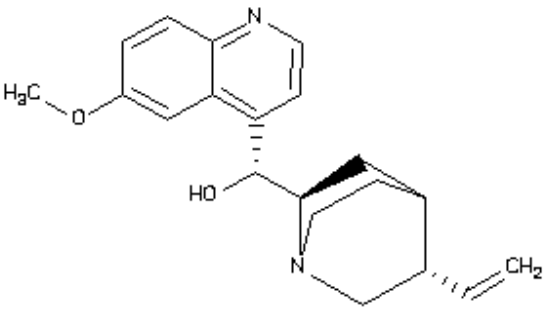


Figure 1: Quinine formula ($C_{20}H_{24}N_2O_2$)

In this experience, quinine has been selected as an analyte, a vegetable alkaloid with a bitter taste and soluble in water that has native fluorescence in acid solution, and which can, therefore, be determined by fluorimetry.

3.- RISK PREVENTION AND WASTE MANAGEMENT

Carefully read the safety data sheets of the reagents used and take special care with sulphuric acid. The waste generated should be disposed of in properly labelled containers in the laboratory.

4.- MATERIAL AND REAGENTS (*see Annex I*)

Reagents: Dissolution of 0.5 M sulphuric acid; quinine sulphate dihydrate (solid) $(C_{20}H_{25}N_2O_2)_2SO_4 \cdot 2H_2O$

Instruments and materials: heated magnetic stirrer, ultrasonic bath,UV-Vis absorption spectrophotometer and spectrofluorimeter.

5.- PROCEDURE

In order to carry out the series of experiments described above, a 200 mg/L quinine solution is first prepared from quinine sulphate (500 mL).

5.1.- Excitation and emission spectra.

Prepare 25 mL of a solution of quinine with a concentration of 5 mg L^{-1} in 0.05 M sulphuric medium and obtain the absorption spectrum in the wavelength range between 220 and 450 nm. The maximum absorption wavelength is then located.

Then, using the maximum absorption wavelength as the excitation wavelength, the emission spectrum is obtained and the maximum emission wavelength is located. Finally, the excitation spectrum is recorded and the maximum excitation wavelength is located.

Three emission spectra are then obtained using three excitation wavelengths (e.g. $\lambda_{\text{maximum excitation}}$, $\lambda_{\text{maximum excitation}}$ - 20 nm and $\lambda_{\text{maximum excitation}}$ + 30 nm) and three excitation spectra using three different emission wavelengths. Interpret the observed differences in the spectra. Finally, select at least two pairs of excitation-emission wavelengths for the next experience.

5.2.- Linearity study

In 25 mL volumetric flasks, 10 solutions of quinine are prepared in 0.05 M sulphuric acid medium in the concentration range 0 to 10 mg L^{-1} (e.g. 0; 0.5; 1; 1.5; 2; 2.5; 3; 5; 8 and 10 mg L^{-1}) and the emission intensity is measured by fixing the previously selected excitation and emission wavelengths (at least two pairs of $\lambda_{\text{excitation}}$-$\lambda_{\text{emission}}$ wavelengths). To calculate the detection and quantification limits, obtain 10 readings of the blank.

Linearity and detection and quantification limits are calculated according to the IUPAC recommendation.

From the results obtained, select the working wavelength pair to carry out the determination of quinine in the sample.

Determination of quinine in tonic water

The sample is a carbonated beverage, so the gas is first removed using an ultrasonic bath. The quinine content in tonic water varies between 50 and 100 mg L^{-1}, therefore it is necessary to make the appropriate dilution in 0.05 M sulphuric medium to obtain sample solutions with an analyte concentration of approximately 1 ppm (prepare a triplicate in 25 mL volumetric volumes).
On the other hand, it is necessary to prepare a series of quinine solutions in 0.05 M sulphuric medium in the linear working range (the solutions of section 5.2 can be used, discarding those that are too concentrated).
The emission intensity of the samples and the standards at the selected excitation and emission wavelengths are then measured, the calibration line equation is calculated and finally, from the sample signals, the concentration of quinine the concentration of quinine in the sample solution and in the tonic water is obtained. Express the results in mg L^{-1}, with a 95% confidence level.

6.- REFERENCES

- A.Maurí, M.Llobat and R.Herraez.*"Laboratorio de Análisis Instrumental"*. Servei de Publicacions de la UV and Editorial Reverté (2010)
- Russo S.F. *"Two fluorescence experiments",* J. Chem Educ. 46(6) (1969) 374-377.
- O'Reilly J.E. *"Fluorescence experiments with quinine",* J. Chem Educ. 52(9) (1975) 610-612.

7.- QUESTIONS

1.- Justify the differences and/or similarities observed between the wavelengths of maximum absorption and maximum excitation.

2.- What differences will be observed in the excitation spectra of quinine when varying the fixed emission wavelength?

3.- What expression relates the emission intensity obtained with the concentration of fluorescent compound? Under what conditions can linear response be expected?

4.- Compare the response lines obtained at two different wavelengths.

5.- Explain in detail how to prepare a 200 ppm quinine solution from quinine sulphate.

8.- ANNEX I

PREPARED SOLUTIONS

- Sulphuric acid 0.5 M

DISSOLUTIONS TO BE PREPARED BY THE STUDENT

- Dissolve 200 ppm quinine from quinine sulphate dihydrate (weigh at least 0.1 g quinine sulphate dihydrate and use a 500 mL graduated flask.

- Quinine standard solution of 50 ppm

- Quinine calibration solutions in 25 mL volumetric flasks.

- Measuring solutions of the sample in 25 mL volumetric flasks.

EXPECTED CONTENT OF THE SAMPLE

- Quinine 50-100 ppm

1.- OBJECTIVE

Carry out the determination of the sucrose content in condensed milk by polarimetry.

2.- BASIS

Compounds that present asymmetry in their molecular structure, as in the case of substances with chiral carbon atoms, are able to rotate the plane of polarized light, and are called optically active substances. Polarimetry is based on the measurement of the angle of rotation (α) of a beam of polarized light when a dissolution of an optically active substance is impacted, which is proportional to the concentration of the dissolved substance (C) and the optical path-length (d):

$$\alpha = [\alpha].d.C$$

where [α] is the specific rotary power of the substance.

Since sucrose is an optically active substance, its determination in condensed milk is usually carried out by polarimetry. In order to do this, the sample must be treated in a suitable way to obtain a clear and transparent liquid on which to measure. In this sense, it is necessary to precipitate or coagulate the proteins and other substances present. Thus, the official method of analysis, based on the standard of the International Dairy Federation, FIL-35:1966, prepares a clear filtrate of the sample by clarifying the sample by successive additions of zinc acetate and potassium ferrocyanide solutions.

It should be noted that sweetened condensed milk contains, in addition to sucrose, other sugars that also have optical activity. This is the case of lactose, which is the natural sugar of milk, which also, in dissolution, presents the phenomenon of mutarotation, a phenomenon that causes variations in the rotational power to reach equilibrium. That is why, in order to encourage and accelerate this phenomenon, it is treated with ammonia. In addition, the sample may also contain what is known as *invert sugar*, which is nothing more than an equimolar mixture of dextrose (glucose) and levulose (fructose) from the natural hydrolysis of sucrose. This process is known as *investment*. Its particular name is due to the fact that sucrose is dextrorotatory (rotates the polarization plane to the right), while inverted sugar is levorotary (rotates the polarization plane to the left), so that a saccharose solution that initially rotates the plane of polarized light to the right, will make it rotate to the left after the hydrolysis process.

The polarimetric determination of sucrose is based on the *Clerget method*, or double polarization, and consists of two stages:

In the first, the reading of the total rotating power of the milk, P_d, is made. This reading is a function of the amount of sucrose contained in the milk *(m·x/100)*, the amount of lactose *(m·y/100)* and the amount of inverted sugar *(m·z/100)*, where *x, y* and *z* are the percentages of sucrose, lactose and inverted sugar, respectively, and *m* is the sample mass. Bearing in mind that the specific rotational powers [α] at 20°C (in

mL dm^{-1} g^{-1}) are 66.5 for sucrose, 52.5 for lactose and -20.2 for invert sugar, the individual reading of a solution containing $m \cdot x/100 \cdot V$ g mL^{-1} of sucrose, $m \cdot y/100 \cdot V$ g mL^{-1} of lactose and $m \cdot z/100 \cdot V$ g mL^{-1} of invert sugar, where *V* is the volume of the sample solution, will be 0.665-d-m.*x/V for* sucrose, $-0.202 \cdot d \cdot m.y/V$ for invert sugar and $0.525 \cdot d \cdot m.z/V$ for lactose, and the total polarimetric P_d reading will be:

$$P_d = 0.665 \cdot d \cdot m \cdot x/V + 0.525 \cdot d \cdot m \cdot y/V - 0.202 \cdot d \cdot m \cdot z/V$$

In the second stage, what is known as the *Clerget inversion principle is carried out*, i.e. a gentle treatment with an acid so that the sucrose is completely hydrolysed, while the lactose and other sugars are practically not hydrolysed.

$$\underset{\text{(sucrose)}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{HCl} \underset{\text{(fructose)}}{C_6H_{12}O_6} + \underset{\text{(glucose)}}{C_6H_{12}O_6}$$

Thus, the polarimetric reading of the rotating power of the milk after the inversion process, P_i, is measured This reading will depend on the amount of lactose (which remains unchanged) and the amounts of invert sugar initially present and that obtained from sucrose. Since one mole of hydrolysed sucrose produces one mole of glucose and one mole of fructose, an amount of 1,000 g of sucrose produces 1,053 g of invert sugar per investment. So:

$$P_i = 1.053 \cdot (-0.202) \cdot d \cdot m \cdot x/V + 0.525 \cdot d \cdot m \cdot y/V - 0.202 \cdot d \cdot m \cdot z/V$$

Considering that for the inversion process an aliquot is taken and acid is added to it to proceed to its hydrolysis, the P_i reading must be corrected for the dilution effect:

$$P_{i\ corrected} = f \cdot P_i$$

Where f is the dilution factor: $f = V_{final}/_{Sample}$.

The difference between P_d and $f \cdot P_i$ directly provides the sucrose content in %, *x*, as the sample mass used is known and the volume in which it has been prepared:

$$x\ (\%) = \frac{V \cdot (P_d - f \cdot P_i)}{0.878 \cdot d \cdot m}$$

where: *d:* length of the cell, in dm; *m:* mass of the sample, in g; *V:* volume at which the sample has been diluted after dissolution, in mL.

However, it should be noted that milk has a high fat and protein content, so that when it is precipitated leave a dissolution volume lower than the capacity performed (V), and should therefore be corrected by subtracting the volume occupied by fat and protein (V).

$$x(\%) = \frac{(V - \Delta V)\cdot(P_d - f\cdot P_i)}{0.878\cdot d\cdot m}$$

The calculation of ΔV is done with the following expression:

$$\Delta V = m\,(1.08\cdot G + 1{,}55\cdot P) / 100$$

where *G* is the percentage of fat in the sample and *P* is the percentage of protein (Nx6.38). These values are usually indicated on the container label, but generically, in whole condensed milk *G* = 9.3% and *P* = 8.8%. Finally, it should also be noted that when working at temperatures other than 20ºC, the value of the polarimeter reading must be corrected. Thus, it has been verified that in the interval 17-23ºC there is a variation of the numerical factor 1/0.878 in 0.386 % per degree of temperature increase. Therefore:

$$x\ (\%)\ \text{corrected} = x + x\cdot 0.386/100\cdot(T-20)$$

where T is the temperature in ºC.

3.- RISK PREVENTION AND WASTE MANAGEMENT

Carefully read the safety data sheets of the reagents used.

The waste must be poured into appropriate containers, which must be properly labelled in the laboratory.

4.- MATERIAL, REAGENTS AND INSTRUMENTATION (*see Annex I*)

Reagents: $Zn(CH_3COO)_2$, $K_4Fe(CN)_6$, phenol red, ethanol, acetic acid, HCl, NH_3

Instruments and materials: polarimeter**,** water bath

Sample: Whole condensed milk

5.- PROCEDURE

5.1.- Preparation of the sample

Carefully mix the contents of the condensed milk can, using a stick. Fat and sugar that may have separated on the lid, bottom or walls must be incorporated perfectly, depending on the position of the can during storage.

All additions of water or reagents must be made in such a way as to avoid the formation of air bubbles and, for the same reason; all mixtures must be made by flask rotation and not by violent agitation.

Weigh as accurately as possible 50 g of the condensed milk using a 250 mL beaker. Add 60 mL of distilled water, at 80-90°C, mix carefully, stir by gentle rotation to avoid foaming, and cool to room temperature. Everything is transferred to a 250 mL volumetric flask. Rinse the beaker with successive amounts of distilled water at 60°C until the total volume is 150 to 190 mL.

Add 5 mL of NH3 1:10, stir and leave to stand for 15 min. Add 3 or 4 drops of 0.2% phenol red aqueous solution and neutralize with 10% acetic acid (pink to yellow change). It's shaken by rotation. Add 15 mL of zinc acetate solution and mix by rotation. Then 15 mL of potassium ferrocyanide solution is added. Mix and make up to the mark with distilled water.

If the presence of air bubbles is observed before reaching the mark, it can be eliminated by printing a rotation movement on the flask. If there is foam, add 1 mL of ethanol before making up to the mark. Shake well and leave the solution to stand for a few minutes.

It is filtered over a dry pleated filter, discarding the first 25 ml of filtrate, until 120 mL of filtrate is obtained.

The polarimetric determination of the sucrose is made on the filtrate. Also **reserve about 40-50 mL of the filtrate for Practice 5** where calcium determination by atomic absorption spectroscopy will be performed.

Polarimetric determination of sucrose.

With the filtrate, the polarimeter tube is filled and the deflection angle, P_d, is read.

To make the inversion, 40 mL of the previous filtrate is placed in a 100 mL beaker, 3 mL of 12 M HCl is added and immersed in a water bath at 60°C for 10 min, shaking a little. Cool to room temperature, transfer the contents to a 50 mL volumetric flask, wash the beaker with small portions of distilled water which are transferred to the flask, and make up to the mark with distilled water.

It is left to rest for one hour. With this solution, the polarimeter tube is filled and a new reading is made. This reading is P_i, and must be corrected by dilution factor *f*.

With the two results, P_d and P_i, the percentage of sucrose in the sample is calculated, applying the Clerget formula.

6.- REFERENCES

- *"Laboratorio de Análisis Instrumental*", A. Maurí, M. Llobat and R. Herraez. Servei de Publicacions de la UV i Editorial Reverté (2010).
- B.O.E. 173, 21 July 1977, p. 16281-16282. Official Methods of Analysis of Oils and Fats, Cereals and Derivatives, Dairy Products and Grape Products, established by Order of 31 January 1977 (continued).
- "*Official Methods of Analysis: Milk and Milk Products*". Panreac Química S.A. (available online)
- "*Statistics and Chemometrics for Analytical Chemistry*", J.N. Miller y J.C. Miller, 6th Edition, Pearson Education Limited, England. 2010 (or any edition).

7.- QUESTIONS

1.- What types of compounds can be determined by polarimetry?

2.- Why, in order to carry out the measurements of the rotary power, is it necessary that the solutions are transparent?

3.- Why is temperature control important during polarimetric measurements?

4.- Deduce Clerget's formula.

5.- What is the mutorotation?

7.- Explain the use of employed reagents in the sample treatment.

8.- ANNEX I

PREPARED SOLUTIONS:

- Potassic ferrocyanide solution (10.6 g $K_4Fe(CN)_6 \cdot 3\ H_2O$ in 100 mL)
- Zinc acetate solution (21.9 g $Zn(CH_3COO)_2$ and 3 mL acetic acid 10 % in 100 mL)
- Phenol red (0.2 % aqueous solution)
- Concentrated HCl
- NH_3 10 % (1:10 from concentrated NH_3)
- Ethanol

1.- OBJETIVE

Determination of calcium in condensed milk, previously deproteinizided, by flame atomic absorption spectrometry.

2.- BASIS

Calcium is one of the main components of milk, which is of vital importance for the constitution and maintenance of the bone skeleton. Its content in milk is very variable, depending on the composition of the pasture soils and their origin (cattle, goats, sheep, etc.). In addition, it is influenced by the various treatments to which milk is subjected (whole, semi-skimmed or skimmed). In addition, the calcium content may be increased in the so-called enriched milks.

The determination of calcium in condensed milk is carried out by means of flame atomic absorption spectrometry technique. It should be noted that the phosphate ion present in the sample may interfere with the determination, due to the formation of calcium phosphate that in the flame gives rise to pyrophosphates, refractory compounds of low atomization performance. However, this can be avoided without the need for prior separation by adding an element that forms a more stable compound with phosphate such as La(III), thus freeing calcium. In addition, La(III), due to its low ionization potential, also acts as an ionization suppressor, decreasing the ionization of Ca in the flame.

On the other hand, the organic matter present in the sample could interfere with the determination, especially in the nebulization process. Therefore, the sample must be previously mineralized (by calcination) or deproteinization must be performed (procedure performed in the previous practice).

3.- RISK PREVENTION AND WASTE MANAGEMENT

Read the reagents safety data sheets carefully. The waste must be poured into the appropriate containers, which will be properly labeled in the laboratory.

4.- MATERIAL, REAGENTS, INSTRUMENTATION

Reagents: $CaCO_3$ (standard reagent), Solution 1% La(III) **(Prepared)**, HCl

Instrument and materials: Flame (air/acetylene) atomic absorption spectrophotometer equipped with a hollow cathode lamp of Ca.

Samples: Condensed milk

5.- PROCEDURE

5.1.- Preparation of standard solution of calcium

Weigh the appropriate amount of $CaCO_3$ (in analytical balance and weighs substances) with the maximum precision and accuracy to obtain 50 mL of a 1000 µg/mL solution of Ca. Dissolve in 10 mL of 1 M hydrochloric acid. Cover with a watch glass and heat to a gentle boil until the removal of CO_2 and its dissolution. Cool to room temperature, heating if necessary and, once cold, transfer the solution to a 50 mL volumetric flask and make up to the mark with the wash waters of the beaker. By proper dilution thereof, 100 mL of 50 µg/mL Ca solution is prepared.

5.2.- Preparation of sample solution

Weigh in analytical balance 2.0000 g of milk in a 50 mL beaker. Add about 20 mL of previously heated deionized water at about 80°C and mix well by rotation. Cool and transfer to a 50 mL volumetric flask. Make up to the mark and label.

5.3.- Determination of calcium by standard addition calibration

Taking into account the linear response interval for calcium (~ 10 µg/mL) and that the condensed milk contains approximately 2 mg of calcium per gram, prepare a series of solutions containing, in 25 mL volumetric flasks:

- 1 mL of the previously prepared milk sample
- Known and variable volumes (v_i) of the standard calcium solution.
- 2.5 mL of the 0.1% lanthanum solution.
- Distilled water. Cover and make up to the mark.

For each solution, measure the absorbance.

5.4.- Determination of calcium by external calibration

Prepare a series of standard calcium solutions with a concentration between 0 and 8 µg/mL in the presence of a concentration of La(III) of 0.1%. The first solution of the series prepared for the standard addition calibration is used as the measurement solution of the sample since it does not contain an added standard (it only has the sample and lanthanum). In 25 mL volumetric flasks, prepare a calibration containing:

- 2.5 mL of 1% solution of La(III)
- Different volumes vi of standard calcium
- Make up to the mark, cover and shake

For each solution, measure the absorbance.

5.5.- Determination of calcium in deproteinization filtrate

As a deproteinized milk solution (obtained in the previous practice) is available, the calcium content is determined in this sample. Thus, we can compare the results of the two procedures (with and without deproteinization) to assess whether the deproteinization introduces any modification as far as the response obtained for calcium is concerned.

The procedure for the preparation of the measurement solution is as follows:

10 mL of the filtrate obtained in the previous practice are taken to 50 mL with deionized water in a volumetric flask. Next, 1 ml of the previous solution, 2.5 mL of 1% La(III) solution and deionized water in a 25 mL are introduced in a volumetric flask. Absorbance is measured.

5.6.- Measurements to perform

Absorbance measurements are made at 422.7 nm by adjusting the zero absorbance with the blank (solution of La(III) 0.1%). Once the absorbance values are obtained, the absorbance of the first point of the addition calibration in the external calibration and the deproteinized solution are interpolated to find the calcium concentration in these solutions. Taking into account the dilutions, the concentration of calcium in the samples (µg/mL calcium) is calculated.

In standard addition calibration, the calcium concentration is obtained by extrapolation in the calibration line. Again, taking into account the dilution, the calcium concentration is found. The final result must be expressed as mg of calcium per gram of milk (mg / g) taking into account the initial mass of weighed sample.

6.- REFERENCES

- Maurí, M. Llobat, R. Herráez, *"Laboratorio de Análisis Instrumenta"l*. Servei de Publicacions de la UV y editorial Reverté (2010)

7.- QUESTIONS

1.- Indicate and justify the need to add La (III) to the measurement solutions.

2.- Why should a sample of milk be treated? Could the calcium content be measured directly without this treatment? Justify the answer.

3.- What type of error is corrected by standard addition calibration?

4.- How could a matrix effect be detected experimentally?

PRACTICE 5: DETERMINATION OF LITHIUM IN WATERS BY FLAME ATOMIC EMISSION: STUDY OF THE INFLUENCE OF VARIABLES ON THE ANALYTICAL SIGNAL

1.- OBJECTIVE

Study of the influence of variables that affect atomic emission. Interferent elimination by precipitation. Determination of lithium in natural water.

2.- BASIS

Lithium is a minor component of minerals and occurs in concentrations less than 0.2 ppm in freshwater, while the content in salt or thermal water may be clearly higher. Lithium can be also found in wastewater from the metallurgical industry or glass manufacturing.

The determination of lithium, similar to that of other alkali metals, can be carried out by atomic emission spectroscopy. Lithium emits at 670.8 nm and Ca, Sr and Ba are spectral interferences that can be eliminated by prior precipitation of these ions.

3.- RISK PREVENTION AND WASTE MANAGEMENT

Carefully read reagents safety data sheets. Wastes must be placed into the appropriate containers, which will be properly labeled in the laboratory.

4.- REAGENTS, MATERIAL, INSTRUMENTATION (*see ANNEX I*)

Reagents: Na_2SO_4, Na_2CO_3, LiCl, $Ca(NO_3)_2$, $Ba(NO_3)_2$, $Sr(NO_3)_2$ $4H_2O$

Instruments and materials: heating plate and flame espectrophotometer , filter paper with pore size 2-5 μm

Sample: carbonated water

5.- PROCEDURE

5.1.- Influence of the presence of calcium, barium and strontium on the analytical signal

Using 25 mL volumetric flasks prepare three series of 0.2 ppm Li solutions containing increasing amounts of calcium, barium and strontium (for example, 0, 100 and 3000 ppm) and 0.2% potassium. In addition, prepare a 1 ppm solution containing 0.2% potassium to configure the monochromator and adjust the photomultiplier power and a blank (0.2% potassium solution).

Measure the emission signal for each solution and from the signals obtained assess the need to eliminate the interference of calcium, barium and strontium by precipitation of carbonates and sulfate.

Table 1: Interferent study

Solution	Li	K	Interferent
1	0.2 ppm	0.2 %	-
2	0.2 ppm	0.2 %	Ca^{2+} 100 ppm
3	0.2 ppm	0.2 %	Ca^{2+} 3000 ppm
4	0.2 ppm	0.2 %	Ba^{2+} 100 ppm
5	0.2 ppm	0.2 %	Ba^{2+} 3000 ppm
6	0.2 ppm	0.2 %	Sr^{2+} 100 ppm
7	0.2 ppm	0.2 %	Sr^{2+} 3000 ppm

5.2.- Lithium determination in waters

Add 2.5 mL of sample into a beaker, then introduce 25 mL of distilled water and 2.5 mL of precipitating solution. The mixture has to be boiled until the volume is reduced to one third of the initial volume. After, allow to stand for 30 minutes and filter with 25 mL volumetric paper. Finally, after washing with water, make up to 25 mL. Prepare the sample in triplicate.
Then, in 25 mL volumetric flasks, prepare calibration solutions between 0 and 0.2 ppm of Li by adding the necessary standard solution volumes and 2.5 mL of precipitating solution.

The most concentrated calibration solution is used to configure the monochromator and adjust the photomultiplier. After, the emission signal of the standard solutions and sample solutions is measured. Next, the calibration curve equation is obtained, the signal from the samples is interpolated and the lithium content is calculated. The lithium content is expressed in ppm with a 95% confidence level.

6.- REFERENCES

- *"Laboratorio de Análisis Instrumental"*, A.Maurí, M.Llobat i R.Herráez. Servei de Publicacions de la UV i editorial Reverté (2010)
- APHA, AWWA y WFCF *Métodos Normalizados para el análisis de aguas potables y residuales*, Ed. Diaz de Santos, Madrid

7.- QUESTIONS

1.- Which interferences do introduce the presence of Ca, Ba and Sr in the determination of lithium?

2.- Why precipitant solution is added to the standards solutions?

3.- If the determination is carried out without eliminating the interference, justify whether the error made will be due to excess or default.

4.- Why are alkaline elements usually determined by emission and not by absorption?

5. The determination of alkali metals, easily ionizable elements, by flame emission spectroscopy requires the use of an ionization suppressor. Is ionization suppressor added in this case? Justify the answer.

8.- ANNEX I

PREPARED SOLUTIONS

- 100 ppm Lithium standard solution ⚠ (write down the real concentration at the laboratory)
- 10000 ppm Ba(II) solution
- 10000 ppm Sr (II) solution
- 10000 ppm Ca(II) solution
- 1% K^+ solution
- Precipitant solution 5 g of Na_2SO_4 and 10 g of Na_2CO_3 in water 1L

SOLUTION TO PREPARE BY THE STUDENT

- Lithium standard solution 1 ppm with 0,2 % K in 50 mL
- Lithium+interferent solution with 0,2% K in 25 mL volumetric flasks
- Lithium calibration solutions with precipitant solution in 25 mL volumetric flasks
- Measurement solutions of the sample

EXPECTED CONCENTRATION IN THE SAMPLE

- 1-2 ppm

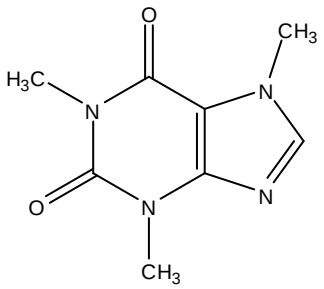
PRACTICE 6: DETERMINATION OF CAFFEINE BY LIQUID CHROMATOGRAPHY

1.- OBJECTIVE

To study the influence of the composition of the mobile phase on the chromatograms obtained by reversed phase high performance liquid chromatography (HPLC) for mixtures of caffeine, aspartame and benzoic acid in order to select the most suitable mobile phase for the determination of caffeine in soft drinks that contain this compound.
To determine caffeine in commercial soft drinks by HPLC.

2.- BASIS

The stimulating power of caffeine, present in a great variety of products, has popularized its consumption even though its ingestion may cause harmful effects on health, more specifically heart problems.



As a result, it is of general interest to determine caffeine in products such as soft drinks, which usually contain appreciable quantities of this compound.

High-performance liquid chromatography (HPLC) can be used to determine caffeine in these samples. Other additives present in beverages, such as saccharin, aspartame or benzoic acid, show similar behaviour, which makes necessary the optimisation of the experimental variables as a previous step to the determination of caffeine in this type of samples.

In this practice the determination of caffeine in different soft drinks samples by high performance liquid chromatography is carried out using a C18 column and a mobile phase of acetonitrile-water with UV/Vis detection. Firstly, working conditions such as the mobile phase composition and pH are optimized. Next, the caffeine content in the samples is determined using the optimum conditions.

3.- RISK PREVENTION AND WASTE MANAGEMENT

- Read the document "**General safety regulations in the laboratory**".
- Check the label of the reagents, in particular those related to safety.

- Dispose the waste generated in the properly labelled containers located in the laboratory.
- The degree of danger of the instrumentation used is low, if used under the appropriate conditions.

4.- REAGENTS, MATERIAL, INSTRUMENTATION (*see ANNEX I*)

Reagents: Caffeine, benzoic acid, sodium aspartame, acetic acid, NaOH, acetonitrile.

Instruments and materials: Solvent filtration equipment and with 0.45 nylon filters µm pore nylon filters, filters for samples, ultrasonic bath, liquid chromatograph equipped with a LiChrosphere RP18 column and a UV-Vis detector.

Sample: Commercial cola soft drinks.

5.- PROCEDURE

5.5.- Optimisation of the mobile phase

To study the influence of the pH of the mobile phase on the retention times of caffeine, benzoic acid and aspartame, inject 20 µL of each of the following solutions:

- 100 ppm caffeine solution
- 100 ppm benzoic solution
- 500 ppm aspartame solution
- Multicomponent solution containing 50 ppm of caffeine, 50 ppm of benzoic acid and 200 ppm of aspartame

using mobile phases with 25% acetonitrile (constant composition) and variable pH. Register the chromatograms at a wavelength of 254 nm, and according to the results obtained select the working pH.

In order to study the influence of the composition of the mobile phase on the retention times, use mobile phases of pH 3 (constant pH) and variable acetonitrile percentages (25% and 15%) in a similar way. Register the chromatograms at a wavelength of 254 nm, and on the basis of the results obtained select the composition of the working mobile phase.

5.6.- Determination of caffeine in the sample

Once the mobile working phase has been selected, five standard solutions of caffeine of concentrations of 20, 40, 60, 80 and 100 mg L^{-1} are prepared in water (in 10 mL volumetric flasks) and chromatographed.

The calibration curve is calculated by plotting the peak area of caffeine against its concentration for each of the injected standards.

The sample solutions are prepared by triplicate. Make the appropriate dilution of the sample taking into account that the approximate concentration of caffeine in it is expected to be 100 mg L^{-1}. Register the chromatograms of the sample replicates under the selected working conditions.

Finally, the content of caffeine in the sample solutions is obtained by interpolating the signals in the calibration curve, and considering the sample dilution carried out, the concentration of caffeine in the original sample is calculated. Express the result as mg L^{-1} with a 95% confidence level.

6.- REFERENCES

- *"Instrumental Analysis Laboratory",* A.Maurí, M.Llobat and R.Herráez. Servei de Publicacions de la UV and Editorial Reverté. (2010)
- "Analytical Separation Techniques", Valcarcel, M., Ed. Reverté.

7.- QUESTIONS

1.- Why it is necessary to degasify the mobile phase and sample before their introduction into the chromatograph?

2.- How does the pH influence the retention times of each of caffeine and benzoic acid? Justify the answer based on their acid-base behaviour.

3.- Justify the effect of the acetonitrile content in the mobile phase.

4.- What differences in the chromatograms can be expected if the detection was carried out at 270 nm?

8.- ANNEX I

SOLUTIONS PREPARED:

- Mobile phases of acetonitrile and acetic-acetate buffer of variable composition:

a) 25% acetonitrile and acetic-acetate buffer of pH 3,

b) 25% acetonitrile and acetic-acetate buffer of pH 5,

c) 15% acetonitrile and acetic-acetate buffer of pH 3

SOLUTIONS TO PREPARE BY STUDENTS:

-100 mL of a caffeine solution of concentration 1000 mg L^{-1}

- 50 mL of a benzoic acid solution of concentration 500 mg L^{-1}

- 50 mL of an aspartame solution of concentration 1000 mg L^{-1}

- 100 ppm caffeine standard solution

- 100 ppm benzoic acid standard solution

- 500ppm aspartame standard solution

- Multi-analyte standard solution: 50 ppm caffeine, 50 ppm benzoic acid, 200 ppm aspartame

- Caffeine calibration standard solutions

- Sample solutions

1.- OBJECTIVE

The objective of this practice is to carry out the determination of a series of phenols of interest in urine samples by gas chromatography. In order to perform a cleaning of the matrix as well as a pre-concentration of phenolic compounds, the solid-phase extraction (SPE) technique will be used.

To carry out this determination, standard solutions and samples will be subjected to the same treatment and the internal standard calibration method will be applied. In addition, the extraction yield will be calculated by comparison of the signals obtained for standard solutions subjected to SPE with those obtained without SPE treatment.

2.- BASIS

Control of workers exposed to organic solvents can be done by phenol analysis. Phenols are metabolites of benzene and its alkyl derivatives are present in both blood and urine.
In the human body, any xenobiotic undergoes phase II metabolism reactions, in which both the starting compound as well as the phase I metabolites that may have been generated are conjugated with polar molecules, such as glucuronic acid, to facilitate urinary excretion. Therefore, in order to determine the total phenol content, it is necessary to carry out a hydrolytic treatment of the sample, in this case urine, in order to hydrolyse the conjugates and release the compounds of interest. This stage can be carried out using concentrated and hot acids (chemical hydrolysis), or by using enzymes such as glucuronidase (enzymatic hydrolysis). In addition, since urine is a fairly complex matrix, a cleaning process is required to remove potentially interfering substances, which can be done by liquid-liquid extraction or solid-phase extraction. Besides, chromatographic separation is necessary prior to detection, for which liquid chromatography with UV/Vis spectrometry detector (LC-UV/Vis) or gas chromatography with flame ionisation detector (GC-FID) is commonly used.
Solid-phase extraction (SPE) is based on the selective retention of analytes present in a liquid sample on an immobilised solid sorbent, usually inside a tubular syringe-shaped cartridge (extraction column).

Once the solid-phase has been conditioned, a known volume of the sample solution is passed through the extraction column by applying a positive pressure, either by vacuum or by gravity, in such a way that the analytes are retained in the sorbent. Other components of the sample may be retained with the compounds of interest. For this reason, working procedures usually incorporate a washing step with a suitable solvent which, ideally, eluates only the undesirable substances from the column. Finally, the analytes are desorbed with a solvent of proper eluent strength, and the collected extract is processed by the chosen technique, in this case by gas chromatography (GC).

In general, in SPE technique, the volume in which the analytes are collected is much lower than the volume of the sample in order to achieve a larger pre-concentration factor. Alternatively, if the compounds of interest are not volatile, the extract obtained may be partially or totally evaporated. Even the extract can be taken to complete dryness, and the residue solved in a smaller volume. The preconcentration factor

can be calculated using the following equation:

$$Preconcentration\ factor = \frac{C_{extract}}{C_{sample}}$$

where $C_{extract}$ is the obtained concentration of the extract and C_{sample} is the initial concentration in sample. The preconcentration factor can be calculated using the obtained signals for the same solution before and after SPE treatment since the signal is proportional to the concentration (within the linearity range).

On the other hand, taking into account that:

$$C_{extract} = \frac{m_{extract}}{V_{extract}}\ and\ C_{sample} = \frac{m_{sample}}{V_{sapmle}}$$

The preconcentration factor can be expressed:

$$Preconcentration\ factor = \frac{C_{extract}}{C_{sample}} = \frac{V_{sample}}{V_{extract}}\ R$$

where R is the recovery of the analyte under extraction conditions. Thus, the SPE allows both purification of sample (removing the matrix components) and preconcentration of the target compounds.

As far as phenol and its derivatives are concerned, the polymeric phases are usually the sorbents that have provided the best preconcentration factors of these compounds. However, C_{18} silica sorbents can allow the determination of the main phenols in specific applications, such as the analysis of industrial effluents and wastewater or biological fluids.

In this practice, the calculation of the extraction yield and the determination of phenols in urine by GC after SPE treatment with C18-modified silica sorbent will be carried out.

3.- RISK PREVENTION AND WASTE MANAGEMENT

- Read the document "**General safety regulations in the laboratory**".
- Check the label of the reagents, in particular those related to safety.
- Dispose of the waste generated, including the extraction columns, in properly labelled containers located in the laboratory.
- Handling urine samples requires extreme caution. It is necessary to use gloves to avoid contact

with the skin and to protect oneself from possible splashes with a mask.

4.- REAGENTS, MATERIAL, INSTRUMENTATION (*see ANNEX I*)

Reagents: HCl, acetonitrile, methanol, o-cresol, p-cresol, 2,4-xylenol, ethylguaiacol

Instruments and materials: Gas chromatograph with FID detector provided with a stationary phase composed of 95% dimethyl-5% diphenylsiloxane, C_{18} extraction cartridges, screw tubes with stopper, plastic syringes and adapters for extraction columns.

Sample: urine

5.- PROCEDURE

5.1.- Treatment of the sample and preparation of calibration solutions

5.1.1.- Preparation of calibration solutions: Using 10 mL-volumetric flasks and the aqueous multicomponent stock solution, prepare multicomponent standard solutions within the concentration range 20-100 mg mL^{-1} in 0.01 M HCl medium. From each of these solutions, 5 mL are taken and transferred to 10 mL screw tubes, where 0.6 g of NaCl is added.

5.1.2.- Extraction performance: Prepare (using a 10 mL graduated flask and the multicomponent stock methanolic solution) a multicomponent standard solution of 50 mg mL^{-1} using as diluent the acetonitrile-methanol mixture (1:10, v/v) containing a concentration of 100 ppm of internal standard. Transfer approximately 1 mL of this solution to an injection vial and seal.

5.1.3.- Sample treatment: The sample is treated in order to determine the total content (free + conjugated) of phenols. 25 mL of urine are taken in triplicate, followed the addition of 2 mL of concentrated HCl. The solutions are heated at 95°C for 90 minutes. Then, they were allowed to cool at room temperature and set to pH 2 with NaOH 1 M, and fill with deionized water up to 50 mL-volumetric flask. **(A hydrolyzed sample is provided to the student)**
From each of these solutions, 5 mL are taken and transferred to 10 ml screw tubes, to which 0.6 g of NaCl are added.

5.1.4.- SPE protocol: In order to carry out the SPE procedure, it is first necessary to activate nine extraction cartridges by flushing through with 5 mL of methanol and then with 8 mL of deionized water, preventing air passing through the column. Then, a blank, the standard solutions and the samples are passed through the extraction cartridges, being the solution remaining in the tube collected with small volumes of 0.01 M HCl (Avoid air passing through the columns).

Then, the cartridges are washed with 2 mL of 0.01 M HCl and air is passed through the cartridges to dry them. The analytes are then eluted using 2 mL of a mixture of acetonitrile:methanol (1:10, v/v) containing a concentration of 100 ppm of internal standard. The extract is collected in a clean and dry tube. Finally, the solutions are transferred to the injection vials and sealed.

5.5.- Obtaining analytical signals

Extracts of the standard and sample solutions and the 50 ppm solution (not subjected to SPE treatment) are injected into the chromatographic system using the following conditions:

Injection volume: 0.5 µL (without split)
Injector temperature: 270 ° C
Temperature program: 50°C (1 min), 5°C / min up to 90°C, 20°C / min up to 120°C (2.5 min)
Detector temperature: 280°C
Carrier gas: Nitrogen at 2.5 mL min^{-1}

Figure 1 shows a chromatogram of the analytes of interest under the chromatographic conditions described above.

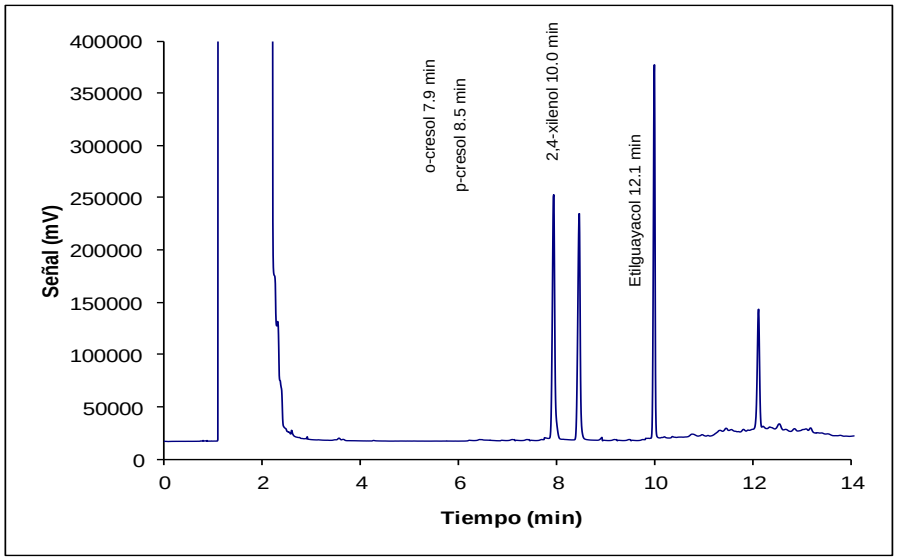


Figure 1. Chromatogram of various phenols of interest obtained under the chromatographic conditions described above.

5.6.- Calculation of extraction yield

The signals obtained for the standard solution prepared in Section 5.1.2 are compared with those obtained

for the extract obtained from the 20 ppm aqueous solution and the extraction yield is calculated.

5.6.- Calculation of the concentration of analytes in the test sample

From the chromatograms of the standard solutions prepared in water and subjected to the SPE protocol, the corresponding calibration lines representing (A_i/A_{PI}) against the analyte concentration are obtained.

Then, from the chromatograms obtained for the sample extracts, the corresponding signals (A_i/A_{PI}) are established, which are interpolated in the calibration curves to find the content of each of the phenols in the sample. Express the final result as mg of analyte/g of creatinine taking into account that urine contains a creatinine content of 0.8 g L^{-1}.

6.- REFERENCES

- *"Laboratorio de Análisis Instrumental"*, A.Maurí, M.Llobat i R.Herraez. Servei de Publicacions de la UV i editorial Reverté (2010)
- Grazyna Bieniek, J. Chromatog B (1996), 682,167-172.

7.- QUESTIONS

1.- Why is the sample treated with hot HCl?

2.- Can SPE extraction be carried out under basic pH?

3.- Why do the SPE cartridges have to be conditioned?

4.- Which are the advantages of using a temperature program in GC?

5.- Based on the structure of the phenolic compounds and their boiling points, explain the retention times obtained.

6.- Suggest an alternative to evaluate the extraction yield and preconcentration factor.

7.- What is the role of internal standard?

8.- ANNEX I

PREPARED SOLUTIONS:

- Multicomponent stock solution in methanol containing 1000 mg/L of o-cresol, p-cresol and 2,4-xylenol. (⚠**TAKE NOTE OF EXACT CONCENTRATION IN THE LABORATORY**)
- Multicomponent stock solution in water containing 1000 mg/L of o-cresol, p-cresol and 2,4-xylenol. (⚠**TAKE NOTE OF EXACT CONCENTRATION IN THE LABORATORY**)
- Internal standard (p-ethylguaiacol) solution (500 mg/L) in methanol.

SOLUTIONS TO PREPARE BY STUDENTS:

- 0.01 M HCl solution.
- Acetonitrile:methanol 1:10 (v/v) solution containing 100 ppm of internal standard, NaCl and ultrapure water.
- Calibration standard solutions.

EXPECTED CONTENT IN SAMPLE: 30- 60 ppm

PRACTICE 8: DETERMINATION OF FLUORIDE IN TOOTHPASTE USING AN ION-SELECTIVE ELECTRODE

1.- OBJECTIVE

This practice proposes the use of direct potentiometry for the determination of fluoride content in commercial toothpaste. Additionally, a series of experiments will be done to emphasize the importance of working conditions such as pH or addition of complexing agents.

2.- BASIS

Nowadays, to prevent the development of caries and to remineralize the lesions produced by it, the inclusion of fluoride in toothpaste is considered an effective method. However, fluoride must be administered in limited quantities to avoid possible negative effects. In this sense, currently, a maximum fluoride content of 0.2% is established in toothpastes, being 0.05% for children products (aged from 3 to 6 years).

Potentiometric methods are based on the measurement of the potential difference between a reference electrode and an indicator electrode immersed in the sample. Membrane electrodes, also referred to as ion-selective electrodes, are commonly used as indicator electrodes, whereas an Ag/AgCl electrode is generally used as reference electrode. In this practice, for fluoride determination, an electrode consisting of a LaF_3 crystal doped with EuF_3 (located at the end of tube) is used. Besides, an internal electrode consists of Ag/AgCl electrode immersed in a sealed internal filling solution (containing NaF of known activity) is also required. The resulting potential difference is related to the activity of fluoride in internal solution through the expression:

$$E = K - 0.059 \log a_F$$

Therefore, the relationship of the obtained signals against the logarithm of the fluoride ion activity for a series of fluoride standard solutions can allow the determination of the fluoride activity in the sample.

However, from a practical point of view, the relationship of the signal versus the logarithm of the concentration is preferred, consequently, to obtain linear calibrations it is necessary that the activity factors remain constant along the concentration range used.

On the other hand, the possible presence of interferences in this determination makes necessary to work in conditions in which their effect is avoided or minimized. In this sense, for the determination of fluoride, it is important to control the pH and also the addition of complexing agents that prevent the formation of fluoride complexes of metallic ions such as Fe (III) or Al(III).

3.- RISK PREVENTION AND WASTE MANAGEMENT

- Carefully read the safety data sheets of the used reagents.
- Dispose generated wastes in properly labelled containers located in the laboratory.
- The hazard level of the used instrumentation, working under the appropriate conditions, is low.

4.- REAGENTS, MATERIAL AND INSTRUMENTATION

Reagents: NaF, NaCl, NaOH, sodium citrate, aluminium nitrate, iron (III) nitrate, HCl, sodium acetate.

Instrumentation and material: Magnetic stirrer, Potentiometer, Reference electrode Ag/AgCl, Fluoride selective electrode, pHmeter.

Sample: toothpaste.

5.- PROCEDURE

First of all, prepare 100 mL of a 1000 ppm fluoride standard solution from NaF.

5.1.- Study of the influence of pH

In 50 mL-volumetric flask, prepare three solutions containing 5 mg L^{-1} of fluoride under a constant ionic strength of 1 M (adjusted with NaCl) at several pH values (obtained by adding HCl or NaOH). Measure the potential difference of these solutions. Compare the results obtained and justify the differences observed.

5.2.- Study of the influence of other ions

In order to carry out this study, the following solutions are prepared in 50 mL-volumetric flask:

a) 5 mg L^{-1} fluoride solution in 0.01 M acetic acid/acetate medium.

b) 5 mg L^{-1} fluoride solution in 0.01 M acetic acid/acetate medium containing an appropriate volume of 10^{-2} M Fe(III) solution to obtain a F^-/Fe (III) molar ratio of 0.1.

c) 5 mg L^{-1} fluoride solution in 0.01 M acetic acid/acetate medium containing an appropriate volume of 10^{-2} M Al(III) solution to obtain a F^- /Al (III) molar ratio of 0.1.

Measure the potential difference of these dissolutions and justify the differences observed.

5.3.- Determination of fluoride in the test sample

Preparation of sample

Weigh, in triplicate, approximately 0.15 g of toothpaste sample and placed in a 250-mL beaker and add 25 mL of the TISAB solution. Then, place it in an ultrasonic bath for 10 minutes, and transfer the solution to a 50 mL-volumetric flask and diluted to the mark with deionized water.

Preparing the calibration line

From a standard fluoride solution of 100 mg L-1, prepare, in 50 mL-volumetric flasks, a series of solutions containing 25 mL of the TISAB solution and fluoride concentrations ranging from 0.5 to 10 mg L^{-1}.

Obtaining signals and calculating fluoride content in the sample

Measure the potential difference of these solutions, obtain the calibration curve and determine the fluoride content in the sample expressing the final result as % w/w.

6.- REFERENCES

Light, T.S y Cappucino, C.C “Determination of fluoride in toothpaste using an ion-selective electrode”, J. Chem. Ed. 52 (1975) 247.

7.- QUESTIONS

1.- Justify the influence of the pH on the signals provided by the electrode.

2.- How does the presence of Fe(III) or Al(III) affect the obtained signal?

3.- Why is TISAB added to calibration standards and sample solutions?

4.- Explain if there are differences between two calibration curves obtained from the same concentration range of fluoride but prepared at different ionic strengths?

8.- ANNEX I

PREPARED SOLUTIONS:

- Solution of TISAB (Total Ionic Strength Adjustment Buffer)
- Solution of NaCl 5 M
- Acetic acid/acetate buffer 0.1 M
- Solution of Fe(III) 0.01 M
- Solution of Al(III) 0.01 M
- Solution of 0.1 M HCl
- Solution of NaOH 0.1 M

SOLUTIONS TO BE PREPARED BY STUDENTS:

- Fluoride standard solution of 1000 and 100 mg L^{-1}.
- Fluoride standard solution of 5 mg L^{-1} for the pH and interference studies.
- Fluoride standard solutions from 0.5 to 10 mg L^{-1} for the calibration curve.
- Sample solutions

1.- OBJECTIVE

In this practice, the electrogravimetric determination of copper in a lead-free copper alloy will be carried out. The educational goal of this practice is to introduce the student to the practical aspects of electrogravimetry including the influence of chemical and/or instrumental variables of this technique.

2.- BASIS

An electrodeposition is a quantitative precipitation in which the components to be determined, generally a cation, is separated by an oxidation or reduction process on an electrode immersed in the sample solution. Generally, a metal deposit is formed on the cathode (reduction), although it can also be deposited as an oxide on the anode (oxidation). To carry out these redox reactions (oxidation at the anode, and a reduction reaction at the cathode), it is necessary the introduction of a pair of electrodes into the sample solution and the application of a potential (voltage) difference between them. Therefore, in one of the electrodes should occur the desired reaction, whereas in the other, an oxidation or reduction that guarantees the current flow. This “auxiliary” reaction usually involves the solvent.

Focusing on the electrodeposition by reduction, for this process, it is necessary that the potential applied to the cathode (electrode connected the negative terminal of the electrical cell) is lower that the so-called equilibrium potential of the considered redox pair. This potential can be estimated using the Nernst equation.

In addition, phenomena such as ohmic drop, concentration polarization and activation overpotential, imply, in many cases, the application of lower potential to that established by Nernst equation. Consequently, the potential applied should be clearly inferior to the so-called equilibrium potential.

In the specific case of brass samples, whose major components are copper and zinc, a potential difference of 3-4 volts is enough to achieve the quantitative reduction of copper. The electrode reactions that take place under the working conditions established in the procedure are the following:

Cathodic reaction: $Cu^{+2} + 2\,e^- \rightarrow Cu$

Anodic reaction: $H_2O \rightarrow 2\,H^+ + \frac{1}{2}\,O_2 + 2\,e^-$

Besides, it should be noted that the electrodeposition is carried out in the presence of nitrate, since it acts by hindering the formation of hydrogen that would prevent the proper deposition of copper in the cathode. Nitrate reduction takes place according to the reaction:

$$NO_3^- + 10\ H^+ + 8\ e^- \rightarrow NH_4^+ + 3\ H_2O$$

However, this reaction has a number of drawbacks. On the one hand, there is a large consumption of electric current (8 moles of e^- for each mole of NO_3^-), and on the other hand, it really occurs in two stages, through the formation of nitrous acid, which decomposes forming toxic oxides of nitrogen:

$$NO_3^- + 3H^+ + 2e^- \leftrightarrows HNO_2 + H_2O$$

$$HNO_2 + 7H^+ + 6e^- \leftrightarrows NH_4^+ + 2H_2O$$

To solve these problems, urea is usually used, which reacts with the nitrous acid formed in the first stage, forming N2 (g) and CO2 (g), preventing the completion of the second reaction, and therefore, avoiding the consumption of e-, as well as the formation of nitrogen oxides.

$$NO_3^- + 3H^+ + 2e^- \leftrightarrows HNO_2 + H_2O$$

$$2HNO_2 + NH_2CONH_2 \leftrightarrows 2N_2\uparrow + CO_2\uparrow + 3H_2O$$

Since N_2 and CO_2 are formed within the solution, the process of copper deposition can be easily accomplished.

3.- RISK PREVENTION AND WASTE MANAGEMENT

- Carefully read the document “General laboratory safety rules”.
- Check the label of reagents, in particular those related to safety.
- Pour the waste generated into appropriate containers, which will be properly labelled and located in the laboratory.
- The hazard level of the instrumentation used in the practice, working in proper conditions, is low.

4.- MATERIAL, REAGENTS, INSTRUMENTATION

Reagents: Sodium nitrate, urea**,** concentrated nitric acid**,** concentrated ammonia

Instruments and materials: 3 magnetic stirrer and heater, 3 power supplies, 3 platinum electrodes, 3 stainless steel electrodes

5.- PROCEDURE

5.1.- Sample preparation

After degreasing the electrodes with ethanol or acetone and drying the sample, approximately 0.5 g of the sample is weighed (with adequate accuracy) in triplicate. Then, the samples are introduced into two beakers and 2 mL of concentrated nitric acid is added to each of them, and subsequently, 15 ml of water. The sample is allowed to digest by heating slightly if necessary. This process is done in a laboratory fume hood. Once the digestion is finished, the resulting solution is boiled (under mild heating) until its volume is reduced to the third part. In any case, check that nitrous vapors are no longer released; otherwise, keep boiling until complete removal.
In presence of tin, a yellowish white precipitate will be formed and it should be removed by filtration.

5.2.- Cathode conditioning

Wash the cathodes with soap and water, rinse with deionized water and then with acetone. Once they are cleaned, avoid touching them with hands. Dry in an oven at 110ºC (about 10 minutes), cool and weigh at room temperature.

5.3.- Cupper electrodeposition

To each sample solution, deionized water is added up to about 100 mL and then 2 g of urea and 2 g of sodium nitrate are added. Then, a magnet is placed in each of the beakers and they are heated, with constant stirring, up to a temperature of 70-80 ° C.
The electrolytic cell is set up by introducing the electrodes into the solution (before the current application), in such way that the anode is located in the central part of the vessel surrounded by the cathode (with cylindrical shape and high surface area).
Once the solution has apparently lost its blue color (due to Cu (II)) it is important to check that the electrodeposition has been quantitative. For that, deionized water is added until the level of the solution is approximately 1 cm above the level of copper deposit, and apply electric current for about 10 more minutes. After this process, check if copper is deposited into the cathode. In the case of a copper deposit is evidenced, this operation should be repeated for 10 in. Otherwise, a few drops of solution are taken in a tube and a few drops of concentrated ammonia are added. If the copper ammonia complexes (deep blue color) are not formed, the process can be considered as completed. Otherwise, electrolysis should be continued for about 10 min.
Once the electrodeposition is finished, the electrodes are removed without disconnecting the power supply, rinsed with water and finally, the electrical supply is disconnected.
The cathodes are washed again with alcohol or acetone, dried in an oven, cooled and weighed.

The increase in weight of each cathode allows establish the calculation of the percentage of copper in the sample.
After weighing the cathodes, the deposits should be removed by immersing them in a 25% (v/v) HNO_3 solution.

6.- REFERENCES

- "Laboratorio de Análisis Instrumental", A.Maurí, M.Llobat and R.Herráez. Servei de Publicacions de la UV and Editorial Reverté (2010).

7.- QUESTIONS

1.- In the procedure described, how is avoided the formation of hydrogen in the cathode? Why is it convenient to avoid hydrogen formation?

2.- What role does urea play in the electrolytic process? Write the reaction that takes place.

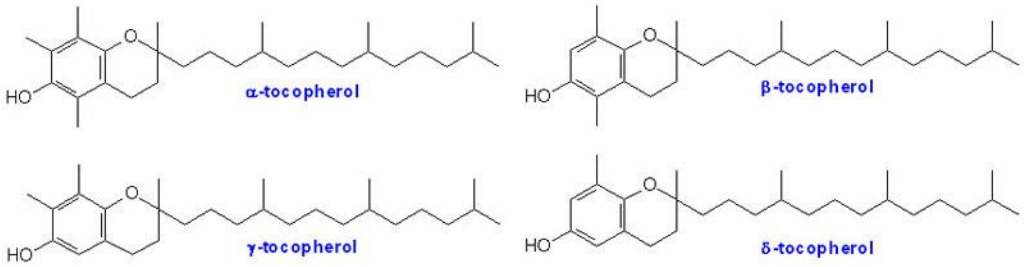
3.- How do you prove that copper reduction has been quantitative?

1.- OBJECTIVE

In this practice, the determination of α-tocopherol content in vegetable oils will be carried out by using differential pulse voltammetry.

2.- BASIS

The α-tocopherol content in vegetable oils constitutes one of the quality parameters of these food products due to the antioxidant role of these compounds. Tocopherols are methyl substituted derivatives of tocol molecule. As can be seen in the figure, the number and position of methyl groups is variable, being possible to distinguish between four different tocopherols. Actually, the name of vitamin E embraces a set of tocopherols with biological activity.



Among them, α-tocopherol shows the largest biological activity, since it reacts with free radicals preventing tissue degradation. Besides, its continued consumption can, in certain way, inhibit the development of cancerous processes. It also seems to influence on the development of cardiovascular or ophthalmic diseases.

Therefore, the consumption of vegetable oils rich in tocopherols is highly recommended by public health agencies.

For the determination of tocopherols in oils, several selective methods have been proposed by using different analytical techniques such as fluorimetry or chromatography.

In this practice, the identification of tocopherols found in a commercial vegetable oil is proposed jointly with the determination of α-tocopherol using differential pulse voltammetry. For this purpose, the analysis of sample is subjected an established potential scan and the height corresponding to the oxidation peak of α-tocopherol is used as analytical signal to perform its determination.

3.- RISK PREVENTION AND WASTE MANAGEMENT

• Carefully read the document "**General laboratory safety rules**".

• Check the label of reagents, in particular those related to safety.

• Pour the waste generated into appropriate containers, which will be properly labelled and located in the laboratory.

• The hazard level of the instrumentation used in the practice, working in proper conditions, is low.

4.- REAGENTS, MATERIAL, INSTRUMENTATION (*see ANNEX I*)

Reagents: Individual standard solutions of α-, γ- y δ-tocopherol, sulfuric acid (96%), ethanol, background electrolyte solution, saturated solution of LiCl in ethanol.

Instruments and materials: Centrifuge, voltammetric analyzer, rotating graphite electrode, platinum electrode, Ag / AgCl reference electrode.

Sample: Vegetable oil

5.- PROCEDURE

5.1.- Sample preparation

Weigh about 0.2 g of sample in two separate centrifuge tubes, and add 10 mL of background electrolyte solution to each tube. After vigorous stirring for 5 minutes, centrifuge the solution for 5 minutes at 3000 rpm. Transfer with the help of an eyedropper pipette, take the supernatant and introduce it into the voltammeter cell.
An identical treatment as described above was also done to prepare a fortified sample with 0.3 mg of α-tocopherol per gram of sample (**already prepared**).

5.2.- Identification of tocopherols in sample

The identification of tocopherols in the sample is carried out based on the voltammograms obtained for the sample and standard solutions of each tocopherol. It important to remark that the voltammogram obtained for a mixture of the four tocopherols has only three peaks, since the peak potentials of β- and γ-tocopherol are identical. In any case, the voltammogram of the sample is recorded using the instrumental conditions indicated in Table 1. Next, additions of each standard tocopherol solutions are made. The volume added in each case must be such that the final concentration added is 10 ppm.
From the resulting voltammograms, the identification of the tocopherols present in the sample is carried out.

Table 1. Working conditions

Voltammetry	
Measuring mode	DP – Differential pulse
Stirring rate	2000 min^{-1}

Potentiostatic pretreatment	
Purge time	60 s
Initial time	15 s
Start potential	550 mV
Equilibration time	30 s
Scanning or Sweep	
Start potential	550 mV
End potential	900 mV
Voltage step	6 mV
Pulse time	40 ms
Potential step time	0.6 s
Scan rate	10 mV/s
Pulse amplitude	50 mV

5.3.- Determination of α-tocoferol in sample

The determination of the α-tocopherol content in the sample is carried out using the standard addition method.

- Under the instrumental conditions indicated in Table 1, firstly, obtain the corresponding voltammogram of blank solution (10 mL of background electrolyte solution).
- Next, obtain the voltammograms after the addition of progressive known volumes of α-tocopherol standard solution of 1000 ppm to the sample.
- From the measurements obtained, the calibration line is constructed and the content in the sample is established giving the final result in mg / g of oil.

5.4- Extraction performance

To evaluate the extraction performance, record the voltammogram of the fortified sample under the instrumental conditions described above, and subsequently, a series of standard solution additions of α-tocopherol are done in a similar way that indicated in the previous section.
From the records obtained, the calibration line is constructed and the α-tocopherol content in the fortified sample is determined by giving the result in mg / g of oil. The extraction yield is calculated from the difference between the concentrations found for the fortified sample and the initial sample.

6.- REFERENCES

- "*Laboratorio de Análisis Instrumental*", A.Maurí, M.Llobat and R.Herraez. Servei de Publicacions de la UV and Editorial Reverté (2010).

7.- QUESTIONS

1.- Represent in a graph the program of applied potential. Indicate in it the pulse amplitude, pulse time, the voltage step and the time between steps.
2.- What is represented in the ordinate axis in a voltammogram obtained by differential pulse voltammetry?
3.- What advantages does the use of standard addition method?
4.- In the proposed experimental procedure, is it important that additions do not involve a considerable increase in volume? Why?

8.- ANNEX I

PREPARED SOLUTIONS:

- Individual standard solutions of α-, β-, γ- y δ-tocopherol 1000 mg/L (⚠**TAKE NOTE OF EXACT CONCENTRATION IN THE LABORATORY**)
- Background electrolyte solution: Add 0.6 grams of sulfuric acid to 50 mL of ethanol (**already prepared**) (⚠**TAKE NOTE OF EXACT CONCENTRATION IN THE LABORATORY**)

SOLUTIONS TO PREPARE BY STUDENTS:
- Sample extracts

EXPECTED CONTENT IN SAMPLE: Check with bibliography

Laboratory Report

1.- Sample information

Reception date:
Date of analysis:
Sample reference:
Physical aspect:
Laboratory reference:
Sampling:

2.- Aim of analysis

3.- Experimental methodology

Analytical technique:
Instruments or equipments used:
Method:

4.- Results

Samples	Replicates	Average	Standard deviation
Sample reference	**1***		
	2		
	3		

***Please indicate if you have rejected some data and the statistical test used for this purpose.

5.- Final results

Average ± $s/\sqrt{n}$

6.- Signature

7.- Annexes

- If necessary, include experimental data and tables or figures related to the study of the influence of variables. Discuss the results obtained.
- Include experimental data used to obtain the calibration curves as well as linear regression equations (data in tables).
- Include data (in tables) concerning to the sample treatment and measurement steps (weight of sample, dilutions made, measured signals, concentrations of the measurement solutions and contents of the sample).
- When appropriate, justify the rejection of experimental data.
- If two different procedures are applied, select the most appropriate and justify the choice by applying some statistical test.