LEARNING UNIT
ANALYTICAL CHEMISTRY III

Study plan		By modules	**Level**	Undergraduate	**Code**	16132
Format	Lecture/lab	**Weekly lecture hours**		2	**Weekly practice hours**	3
Total hours		85 34 theory 51 practice	**Credits**	8	**Module**	Analytical Chemistry and Toxicological Evaluation
Area		Common and basic			**Semester**	5th.
Study Plan		By Modules				
Prerequisites		Analytical Chemistry II				
General objective		Students will be able to apply the principles of each instrumental technique when carrying out their experimentations. They will be able to systematize the data through structured ideas and concepts both orally and in written form. They will apply critical, analytical and synthetic reasoning to select the separation methods and electroanalytical techniques according to the sample analyte.				

Theoretical Content	
Unit 1: SEPARATION METHODS AND THEIR SIMPLE CHEMICAL EQUILIBRIA	
Specific objective: Students will be able to recognize the operations used in the separation methods and distinguish between simple chemical equilibria.	
1.1 Main operations used in separation methods. 1.1.1 Physical methods of separation (decantation, filtration and centrifugation). 1.1.2 Physicochemical methods (discoloration, crystallization and sublimation). 1.2 Simple chemical equilibria as separation methods. 1.2.1 Precipitation 1.2.2. Liquid-liquid extraction 1.2.3. Ionic exchange	1 session 1 hour
Unit 2: INTRODUCTION TO CHROMATOGRAPHY	
Specific objectives: Students will be able to understand the concept of chromatography, mobile phase, and stationary phase. Students will be able to classify the different types of chromatography and to explain the principles that originate chromatographic separations.	
Topics 2.1 Introduction and general theory 2.1.1 Concept of chromatography 2.1.2 Classification of the different types of chromatography 2.1.3 Principles that cause chromatography separations	2 Sessions: 3 hours 2

Unit 3: COLUMN AND PLANAR CHROMATOGRAPHY	
Specific objective: Students will be able to mention the column and planar methodologies of chromatography separations to separate and identify substances.	
Topics 3.1 Classic column and planar chromatography. 3.1.1 How column chromatography works. 3.1.2 Planar chromatography on paper 3.1.3 Planar chromatography on a thin layer.	1 Session 2 hours
Unit 4: CHROMATOGRAPHIC PARAMETERS	
Specific objective: Students will be able to know, distinguish, and effectively apply quantitative and qualitative methods.	
Topics 4.1 Qualitative and quantitative parameters in chromatography. 4.1.1 Qualitative parameters (tr, tm, tr`, k, □, R, HEPT, W, $W_{1/2}$) 4.1.2 Van Deemter equation. 4.1.3 Quantification methods. (NA, EE, EI, NAFR)	3 Sessions: 5 hours
5. LÍQUID CROMATOGRAPHY	
Specific objectives: -Students will be able to understand the fundamentals of liquid chromatography and identify the basic elements of a liquid chromatograph, its characteristics, and variants to choose the correct sample. -They will be able to differentiate normal phase from reversed phase.	
Topics 5.1 Fundamentals of Chromatography of liquids. 5.2. Instrumentation and its characteristics (HPLC) 5.3 Normal and reversed phases. 5.4 Selection of the mobile phase, chromatographic column and analysis detector, depending on the purpose of the analysis and the sample.	3 Sessions: 5 hours 3
6. GAS CROMATOGRÁPHY	
Specific objective: Students will be able to understand the fundamentals of gas chromatography and distinguish each one of the components of the gas chromatograph, their characteristics, and variants in order to use the most appropriate ones according to the purpose of the analysis and the sample.	
Topics 6.1. Fundamentals and classification of gas chromatography. 6.2 Instrumentations and their characteristics. 6.3 Selection of the adequate carrier gas, type of column and detector according to the sample.	3 Sessions: 5 hours 3
7. OTHER CHROMATOGRÁPHIC MÉTHODS	
Specific objective: Students will be able to differentiate liquid and gas chromatography from other chromatographic methods (supercritical fluids, ultra-resolution, micro extraction in solid phase, and coupled methods).	

Topics 7.1 Chromatography through supercritical fluids (SCF) 7.2 Ultra performance liquid resolution Chromatography (UPLC) 7.3 Solid phase micro extraction (SPMF) 7.4 Coupled methods	2 Sessions: 3 hours
Theoretical content	
Unit 8: INTRODUCTION TO ELECTROCHEMISTRY	
Specific objectives: Students will be able to distinguish the basic concepts of electrochemistry as well as to categorize the type of cells and electrodes. They will be able to represent the analytical applications of the Nernst equation.	
Topics 8.1. Fundamentals of electrochemistry 8.2. Types of electrochemical cells. 8.3. Classification of electrodes 8.4. Standard potential 8.5. Nernst equation	2 Sessions 4 hours
Unit 9: POTENTIOMETRY	
Specific objectives: Students will be able to understand how ion selective electrodes work in analytical chemistry. They will be able to solve analytical applications with the Nernst equation through direct potentiometric measurements.	
Topics 9.1. Indicator electrodes 9.2. Direct potentiometry 9.3. Ion selective electrodes 9.4. Applications of the direct potentiometry	3 Sessions 3 hours
Unit 10: POTENTIOMETRIC TITRATIONS	
Specific objectives: Students will be able to understand the process of potentiometric titrations. They will be able to solve analytical applications of potentiometric titrations.	
Topics 10.1. Fundamentals 10.2. Methods of endpoint detection. 10.3. Classification of potentiometric titrations. 10.4. Acid-base titrations (*potentiograms, acid mixture, pka, pkb, spreadsheets*) 10.5. Redox titrations	3 Sessions 3 hours
11. Voltammetric techniques	
Specific objective: Students will be able to understand the fundamentals of amperometry and amperometric techniques. They will understand the applications of Karl Fischer method and its relationship with amperometry.	
Topics 11.1 Fundamentals of amperometry 11.2 Classification of the amperometric titrations	2 Sessions: 2 hours

11.3 Karl Fischer Method	
12. ELECTROPHORETIC METHODS	
Specific objective: Student will be able to understand and distinguish between the fundamentals and applications of electrophoretic techniques (capillary zone, isoelectric focusing, and bidimensional).	
Topics 12.1 Fundamentals of electrophoresis 12.2 Electrophoresis in polyacrylamide and agarose. 12.3 Capillary and isoelectric focusing electrophoresis. 12.4 Bidimensional electrophoresis	2 Sessions: Hours per week 3 hours

Bibliography

Basic:

Harris, D. C. (2007). *Análisis Químico Cuantitativo* 3rd edition in Spanish, Spain, Barcelona: Revertè. S.A

Valcárcel, C. M. and Gómez, H. A. (1994). *Técnicas Analíticas de Separación*, Spain, Barcelona: Revertè. S.A.

Skoog, D., West, D., *et al* (2014). *Fundamentos de Química Analítica*, Mexico. CENGAGE Learning

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Muñoz,C et al. (2015) *Manual de Practices de Electroquímica Analítica y Cromatografía*. *Practices. Potenciometría Directa* (pp. 26-29) Universidad de Guadalajara. Mexico.

Complementary:

Vogel, A. I., &Mendham, J. (2000). *Vogel's textbook of Quantitative Chemical Analysis*. (pp. 161-172) Chicago: Ed. Harlow Prentice Hall.

Ayres, G. H. (1970). *Análisis Químico Cuantitativo*. (pp. 188 - 192). Mexico: Harlam.

Domínguez X. A. (1975). *Cromatografía en papel y en capa delgada*..*Monografia 16 del Programa Regional de Desarrollo Científico y Tecnológico*, (pp1-79). Monterrey Nvo. León. Mexico: Editor Eva V.Chesneau.

Waters. Retrieved on July 25 2015, from http://www.waters.com/waters/es_MX/HPLC---High-Performance-Liquid-Chromatography-Beginner%27s-Guide/nav.htm?cid=10048919&locale=es_MX

Agilent. Retrieved on July 25, 2015 from http://www.chem.agilent.com/en-US/Products-Services/Instruments-Systems/Liquid-Chromatography/Pages/default.aspx#

Phenomenex. Retrieved on July 25, 2015 from http://www.phenomenex.com/sample-preparation.

http://goldbook.iupac.org/L03587.html (Retrieved in November 2015).

Practice program			
Practice No. 1	**Topic: Thin-layer chromatography**	**Length: 5 h** **Number of sessions per week: 2**	

Objective:

Students will be able to know the thin-layer separation technique and apply it in the separation of artificial colors in candy to determine if these colors are allowed in the food industry.

Reagents, material and equipment/ /instruments for this practice:

Reagents:

* Silica gel G 60.
* Methanol.
* Colorants (15 mg/10 mL).
* Phenol-Water (70-30), pour into a development chamber the required amount to have a height between 0.5 and 1.0 cm.

Material:

* Test tube 15 mL.
* Test tube tongs.
* Graduated pipettes 5 mL.
* Glass rod.
* Water bath.
* Glass capillaries
* Glass plates.

Equipment/Instruments:

* Stove.
* Top loading balance.
* Centrifuge.
* Heating plate.
* Development chamber.
* Necessary equipment for thin-layer chromatography.

Applied methods:

Weigh, extract the sample, separate through decantation and centrifuge. Concentrate the solution through water bath evaporation.

Place a portion of each colorant on the thin layer plate.

Place the plate in the development chamber to separate.

Evaluation method:

Mandatory attendance to the practices. If the student is not present, he will not obtain a grade for that (those) practice (s).

Answer the prerequisites of the practice; work with his/her teammates, wash the material and clean the table.

Turn in the practice report with the established format and on the assigned due date.

Safety measures and occupational health:

Follow the safety rules of the lab, the MSDS sheets, and the procedures.

Disposal of physical, chemical and biological waste:

Depending on the case, they need to be classified, neutralized, discarded and/or stored where the

authorities of this campus (CUCEI) designated for their further collection.

Practice No. 2	Topic: Liquid chromatography.	Length: 5 h Number of sessions per week: 2	

Objective:

Students will be able to observe the use of the liquid chromatograph and to know its operational variables in order to get familiar with the use of the device.

They will be able to know the methodology to prepare a test sample before the instrumental analysis in order to identify and quantify the desired component through HPLC, using the external standard technique. In order to do this, students will propose an over the counter drug that contains the component to evaluate.

Reagents, material and equipment /instruments for the practice:

REAGENTS:

* HPLC grade water.
* HPLC grade methanol.
* Q.P. acetaminophen
* Solutions:
    * 2 ppm of acetaminophen
    * water: methanol 50:50

MATERIAL:

* Mortar
* Volumetric flasks and pipettes.
* Micro syringe**.**

EQUIPMENT/INSTRUMENTS:

* Centrifuge
* System to obtain chromatographic grade water.
* Vacuum filtration system.
* Analytical balance.
* Chromatographic pump.
* Manual injector.
* UV/Vis detector
* Computer
* Printer

Applied method:

An adsorbent (stationary phase) is selected. It could be silica gel (SiO_2) or alumina (Al_2O_3), both in a polar character. It is used to separate relatively apolar compounds.

A sample that contains several components with different polarity can be separated to identify and quantify its components if it is introduced inside a column that contains a non-polar stationary phase. If it is later eluted with a polar solvent, the related components will emerge first and the ones compatible in the stationary phase will emerge later. Before the actual practice, it is important to perform the necessary calculations for the preparation of the solutions.

Preparation of the test sample

Get the HPLC ready and record the work parameters.

Inject; print the chromatogram.

Evaluation method:
Mandatory attendance to the practices. If the student is not present, s/he will not obtain a grade for that (those) practice (s).
Answer the prerequisites of the practice; work with his/her teammates, wash the material and clean the table.
Turn in the practice report in due time and manner.

Safety measures and occupational health:
Follow the lab rules, MSDS safety sheets, and procedures.

Disposal of physical, chemical and biological waste:
Depending on the case, they need to be classified, neutralized, discarded and/or stored where the authorities of this campus (CUCEI) designated for their further collection.

Practice Program

Practice No. 3	**Name of practice: "Identification and quantification of caffeine in different drinks using high performance liquid chromatography" (HPLC)"**	**Length: 5 h** **Number of sessions per week: 2**

Objectives
Students will be able to observe the use of the liquid chromatograph and to know its operational variables in order to get familiar with the use of the device.
They will be able to know the methodology to prepare a test sample before the instrumental analysis in order to identify and quantify the desired component through HPLC using the internal standard technique. To do this, students will propose a drink that contains the component to evaluate.

Reagents, material and equipment /instruments for the practice:
REAGENTS:
* HPLC grade water.
* HPLC grade methanol
* Q.P. acetaminophen
* Q.P. Caffeine
* Solutions:
* 2 ppm of caffeine
* 2 ppm de acetaminophen
* water : methanol 50:50

MATERIAL:
* Mortar
* Volumetric flasks and pipettes.
* Micro syringe.

EQUIPMENT/INSTRUMENTS:
* Centrifuge
* System to obtain chromatographic grade water.
* Vacuum filtration system.
* Analytical balance.
* Chromatographic pump.
* Manual injector.
* UV/Vis detector

* Computer
* Printer

Applied method:

Apply the HPLC chromatographic technique to prepare and handle the test sample and to quantify the analyte.

Follow the internal standard technique, which consists of using an internal pattern. In this technique, the sample and the internal pattern are run together in one single injection in the liquid chromatograph.

Evaluation method:

Mandatory attendance for the practice.

Report with the results of the caffeine quantification and the interpretation of the chromatograms.

Questionnaire.

Safety measures and occupational health:

Follow the safety rules of the lab and the MSDS sheets about the used chemical substances.

Disposal of physical, chemical and biological waste:

The used solvents are recovered through distillation, and the waste that cannot be neutralized nor recovered to be re used is separated and stored temporarily for their further collection and disposal according to the environmental regulations.

Practice No.4	**Name of practice:** **"Separation, Identification and quantification of a mixture of alcohols through gas chromatography."**	**Length: 5 h**
		Number of sessions per week: 2

Objectives:

Students will be able to know the components and the operation variables.

They will be able to identify and quantify the components of a test sample in order for the students to become familiar with the use of the instruments and their different applications.

Reagents, material and equipment /instruments for the practice:

REAGENTS:

* Various solvents (Compounds A, B and C)

MATERIAL:

* Volumetric pipettes.
* Test tubes with cap.
* Pipette knob.
* Micro syringe

EQUIPMENT:

* Gas chromatograph.

Applied method:

The technique used is gas chromatography. This technique is used to separate and analyze mixtures of volatile substances. The sample is vaporized and introduced into the flow of a gas, which runs through a column where the components of a mixture are separated. The components that migrate through the column are separated, based on their solubility on the stationary phase and their volatility. They leave the column and are dissolved in the carrier gas. The chromatogram is generated at the moment of plotting the electric signal created by the detector when each substance flows in terms of time.

The technique of normalization of areas with response factors calculates the %V of each component in the sample to analyze.

Evaluation method:
Mandatory attendance for the practice.
Report with the results of the %V quantification of each component of the sample to analyze and interpretation of the chromatograms. .
Questionnaire.

Safety measures and occupational health:
Follow the safety rules if the lab and the MSDS sheets of the chemical substances that were used.

Disposal of physical, chemical and biological waste:
The solvents that were used are recovered through distillation, and those wastes that cannot be neutralized nor recovered to be re used are separated and stored temporarily for their further collection and disposal according to the environmental regulations

Practice No.5	**Name of practice: "Determination of methanol in a test sample through gas chromatography"**	**Length: 5 h**
		Number of sessions per week: 2

Objectives:
Students will be able to apply the gas chromatography to separate, identify and quantify the methanol in a test sample based on its polarity. They will be able to prove that the handling of the gathered data through the gas chromatography and through the liquid chromatography is very similar.

Reagents, material and equipment /instruments for the practice:
REAGENTS:
* Grade HPLC solvents
MATERIAL:
* Volumetric pipettes.
* Test tubes with cap.
* Pipette knob.
* Micro syringe.
EQUIPMENT:
***** Gas chromatograph.

Applied method:
The technique to be used is gas chromatography. This technique is used to separate and analyze mixtures of volatile substances. The principle of the technique is based on the separation of the components of a sample through the differences in their polarity.
The sample is introduced directly as a gas, or if it is introduced as liquid, it will be taken to a temperature that transforms it into a gas in the injector. It will then go into a column that contains a stationary phase and in this case polar through an inert carrier gas. The components related to the stationary phase will emerge at the end and the incompatible ones will emerge faster.
It is important to consider the additional kinetic mobility that arises from the temperature effects, according to the volatility of each component.
In a few words, this is the principle of separation through the technique of gas chromatography.
With the internal standard technique, it is possible to calculate the %V of each component of the analyzed sample. It is necessary to use a reference standard similar to the target components as a witness.

Evaluation method:
Mandatory attendance for the practice.

Report with the results of the %V quantification of each component in the analyzed sample and interpretation of the chromatograms.
Questionnaire.

Safety measures and occupational health:
Follow the safety rules of the lab and the MSDS sheets of the used chemical substances.

Disposal of physical, chemical and biological waste:
The solvents that were used are recovered through distillation, and those wastes that cannot be neutralized nor recovered to be re used are separated and stored temporarily for their further collection and disposal according to the environmental regulations.

Practice No.6	**Name of practice**: **"Potentiometric determination in fluorides of a mouthwash. Ion selective electrode method."**	**Length: 5 h**
		Number of sessions per week: 2

Objectives:
Students will be able to build a calibration curve to quantify the concentration of fluorides in a sample of mouthwash using an ion selective electrode.

Reagents, material and equipment /instruments for the practice:
REAGENTS:
*** Standard solution of ion fluoride 0.1 mg/mL.**
Dissolve 221.0 mg of sodium fluoride (dried at 105°C for 2 h) bring to 1L volume
*** TISAB II preparation**
Pour 500 mL of water in a 1L glass. Add 57 mL of glacial acetic acid, 58 g of NaCl and 4,0 g of CDTA. Stir to dissolve. Place the glass in cold-water bath and adjust the pH of the solution to between 5.0 and 5.5 slowly adding NaOH 6 N (125 mL approximately) and stirring. Place it in a 1L volumetric flask and bring to volume with water. This preparation may also be purchased.
MATERIAL:
***** Potentiometer with fluoride selective electrode and reference electrode.
* Plastic volumetric flask of 25 mL.
* Standard solution of fluoride of 25 mg/L, prepared from NaF (**CAUTION: VERY TOXIC**).
* TISAB solution (Total ionic strength adjustment buffer)
* Plastic pipettes**.**
EQUIPMENT:
*** Fluoride selective electrode** (crystalline mineral membrane), whose sensitive element is a lanthanum trifluoride monocrystal that lets the fluoride atoms move freely in the lanthanum atom net. The solid version of the fluoride electrode (solid electrolyte with no dissolution consists of a silver wire AgF and LaF_3).

Applied method:
The specific electrode consists of a lanthanum fluoride membrane and an internal reference joined by an epoxy body. The crystal is an ionic conductor in which only the fluoride ions move. When the membrane touches a fluoride solution, an electrode potential develops through the membrane. This potential depends on the level of free fluoride ions in the solution and it is measured against a constant external reference potential in a specific ion meter.
The fluoride selective electrode is widely used in the determination of fluorides in a great variety of materials. An essential requirement to use it appropriately is the use of a total ionic strength adjustment buffer **(TISAB)** to adjust all the standards and test samples to practically the same ionic force. This reagent is

used to measure the concentration of fluorides more than their activity.

Evaluation method:
Mandatory attendance for the practice.
Turn in the report with the results of the quantification of the ion fluoride mg/L in the analyzed sample and the interpretation of the calibration curve.
Turn in also the equation of the straight line through the linear regression method, scheming the measured potential (E) versus log [F^-].
Answer a questionnaire.

Safety measures and occupational health:
Follow the safety rules of the lab and the MSDS sheets of the used chemical substances.

Disposal of physical, chemical and biological waste:
The used solutions are separated and stored temporarily for their further collection and disposal according to the environmental regulations.

Practice No.7	**Name of practice: "Acid-base potentiometric titrations"**	**Length: 5 h**
		Number of sessions per week: 2

Objectives:
Students will be able to carry out the potentiometric titration of various acids, observe their ionic behaviors, and use the neutralization graphs to quantity them.

Reagents, material and equipment /instruments for the practice:
REAGENTS:

1. Titrated solution of NaOH 0.1 N. Weigh 4 g of NaOH, water down to 1 L and titrate with potassium biphtalate. (Use CO_2 -free distilled water).
2. 0.1 N of HCl solution. Take 8.6 mL of HCl Q.P. and water down to 1 L.
3. 0.1 N in CH_3COOH solution. Take 5.7 mL of acetic acid Q.P. and water down to 1 L.
4. 0.1 N in H_3PO_4 solution. Take 3.27 mL of phosphoric acid Q.P. and water down to 1 L.

MATERIAL:
* Common use lab material.
* Burette**.**

DEVICES:
* Magnetic stirrer.
* Potentiometer with adequate electrodes (glass-calomel).

Applied method:
The technique applied is the volumetric technique of titration with the final detection of the equivalence point through an indicator electrode and its corresponding potentiometric graph.
From the equivalent volume it is possible to obtain the concentration of the test sample and the value of the pKa or pKb.

Evaluation method:
Mandatory attendance to the practice.
Report with the results of the content quantification of the analyzed samples; the characterization as acid or base (weak, strong, polyprotic, etc.) in due time and manner.
Turn in the prerequisites, calculations and annexes (graphs, photographic evidence and/or flowcharts, etc.), and research homework requested in the workbook.

<table>
<tr><td colspan="3">Organization, performance and good lab practices.</td></tr>
<tr><td colspan="3">Safety measures and occupational health:
Follow the safety rules of the lab and the MSDS sheets of the used chemical substances.</td></tr>
<tr><td colspan="3">Disposal of physical, chemical and biological waste:
The used solutions are separated and stored temporarily for their further collection and disposal according to the environmental regulations.</td></tr>
<tr><td rowspan="2">Practice No. 8</td><td rowspan="2">Name of practice:
“Potentiometric titration of chlorides in hot sauce.”</td><td>Length: 5 h</td></tr>
<tr><td>Number of sessions per week: 2</td></tr>
<tr><td colspan="3">Objective:
Students will be able to determine qualitatively and quantitatively the chloride ion that is present in a bottle of hot sauce through a silver indicator electrode or a silver-silver chloride electrode.</td></tr>
<tr><td colspan="3">Material:
* Common use lab material.
* Burette.
* Magnetic stirrer
* Potentiometer with adequate electrodes (silver-calomel).
* Salt bridge.</td></tr>
<tr><td colspan="3">Applied method:
The technique applied is the volumetric technique of titration with the final detection of the equivalence point through an indicator electrode and its corresponding potentiometric graph.
From the equivalent volume, it is possible to obtain the concentration of chlorides in the test sample.</td></tr>
<tr><td colspan="3">Evaluation method
Mandatory attendance to the practice.
Turn in the report with the results of the chloride content quantification of the analyzed samples.
Turn in the prerequisites, calculations and annexes (graphs, photographic evidence and/or flowcharts, etc.), and research homework requested in the workbook.
Organization, performance and good lab practices.</td></tr>
<tr><td colspan="3">Safety measures and occupational health:
Follow the safety rules of the lab and the MSDS sheets of the used chemical substances.</td></tr>
<tr><td colspan="3">Disposal of physical, chemical and biological waste:
The used solutions are separated and stored temporarily for their further collection and disposal according to the environmental regulations.</td></tr>
<tr><td rowspan="2">Practice No. 9</td><td rowspan="2">Name of practice:
“Determination of humidity using the Karl – Fischer Method.”</td><td>Length: 5 h</td></tr>
<tr><td>Number of sessions per week: 2</td></tr>
<tr><td colspan="3">Objectives:
Students will be able to know the Karl – Fischer Method to determine humidity in a fast and precise way.
They will also be able to use the amperometric method to detect the end-point.</td></tr>
<tr><td colspan="3">Reagents, material and equipment /instruments for the practice:
REAGENTS:
* Karl – Fischer reagent. It is better to buy it already prepared.
* Anhydrous methanol solution. Keep the commercial product at least 24 hours in CaO (freshly calcined),</td></tr>
</table>

CaCl2, Drierite, Mg, etc.

* Standard methanol solution. Measure 0.50 mL of distilled water and water down to 500 mL with anhydrous methanol. 1 = 1 mg of H_2O .

MATERIAL:

* Common use lab material.
* Burette**.**

EQUIPMENT:

* Potentiometer with 2 polarized platinum electrodes.

Applied method:

Evaluation of the Karl-Fischer reagent with a standard solution of methanol and evaluation with sodium tartrate salt. Determination of humidity in a test sample. a) Solid sample (balanced feed, inorganic salt, or hydrated organic salt, etc) and b) liquid sample (solvent, oils, etc.)

The Karl Fischer equipment functions under the electrochemical technique of the amperometric evaluations. An important use of this equipment is the titration of water with the Karl Fisher reagent. The electrodes of the equipment are placed inside the chamber that contains the sample and a non-aqueous solvent, usually anhydrous methanol. The Karl-Fischer reagent is then added through a burette. When the reagent has consumed all the water, the depolarization of the electrodes takes place, and thus strong current flows through them during a period of time.

Evaluation method

Mandatory attendance to the practice.
Report with the results of %humidity quantification of the analyzed samples in due time and manner.
Turn in the prerequisites, and research homework requested in the workbook.
Organization and good lab practices.

Safety measures and occupational health:

Follow the safety rules of the lab and the MSDS sheets of the used chemical substances.

Disposal of physical, chemical and biological waste:

The used solutions are separated and stored temporarily for their further collection and disposal according to the environmental regulations.

Practice No. 10	**Name of practice: "Electrophoretic methods of separation."**	**Length: 5 h** **Number of sessions per week: 1**

Objective:

Students will be able to know the unidimensional electrophoresis technique in polyacrylamide gels to analyze the purity and determine the molecular weight of a candidate protein.

Reagents, material and equipment /instruments for the practice:

REAGENTS:

* Protein extracts from different sources.
* Acrylamide/bis-acrylamide solution 30 % (29:1)
* Tris base 50 mM pH 8.0
* SDS
* Glycerol
* Bromophenol blue
* Ammonium persulfate.

* TEMED
* Coomassie blue.
* Standard proteins with a known molecular weight.

MATERIAL:

* Common use lab material.
* Scissors
* Eppendorf Tubes
* Latex gloves
* Micropipettes
* Microwave resistant plastic containers.

EQUIPMENT:

* Orbital stirrer.
* Microfuge
* Electrophoresis equipment for mini gels.
* Power source
* Dry block

Applied method:

A controlled electric current is used in order to separate biomolecules according to their size and electric charge through a gelatinous matrix. When a mixture of ionized molecules with net charge are placed in an electric field, they exert an attraction force towards the opposite charge pole. After some time, the positively charged molecules will move towards the cathode (negative pole) and those charged negatively will move towards the anode (positive pole).

Preparation of the polyacrylamide gel in a 12% concentration.
Sample charging and scattering of the denaturant gel in constant voltage.
Gel retrieval and preparation for coomassie blue staining.
Discoloring of gel through aqueous system and microwave heating.
Visualization and analysis of the electrophoretic pattern.

Evaluation method

Mandatory attendance to the practice.
Report with the results of the electrophoretic sample of the analyzed samples in due time and manner.
Determination of a molecular weight of a determined protein.
Turn in the prerequisites, and research homework requested in the workbook.
Organization and good lab practices.

Safety measures and occupational health:

Follow the safety rules if the lab and the MSDS sheets of the used chemical substances.

Disposal of physical, chemical and biological waste:

The used solutions that are separated and stored temporarily for their further collection and disposal according to the environmental regulations.

Bibliography for practices

Basic:

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Complementary:

Farmacopea de los Estados Unidos Mexicanos, (2008) Secretaría de Salud.

Proyecto de Norma Oficial Mexicana PROY-NOM-218-SSA1/SCFI-2002, Productos y servicios. Bebidas no alcohólicas, sus congelados y productos concentrados para prepararlas. Especificaciones sanitarias. Información comercial, Diario Oficial de la Federación, 2003.

Grading criteria

Departmental exam:	30 %
Partial exams:	30 %
Homework:	5 %
Practical test:	10 %
Practice reports	25 %
Number of…	
Departmental exams:	1
Partial exams:	2
Practical test	1
Practices:	10

Knowledge, aptitudes, attitudes, values, skills and abilities to develop:

Aptitudes and attitudes:
Analytical, participative, and cooperative.

Values and abilities:
Responsibility, honesty, professional ethics, and environmental awareness.

Skills:
Distinguish the different instrumental techniques and the basic components of the used instruments and equipment.
Explain and apply the principles of the different instrumental techniques.
Carry out the adequate calculations to quantify and identify the target components in different samples.
Handle the lab equipment and material skillfully and responsibly.

Professional fields of application: Physical Chemistry, General Chemistry, Organic, Inorganic, Environmental, Pharmaceutical, Forensic, Clinical and Food Chemistry.
Professor's profile: Service attitude and willingness to approach students as well as knowledge of instrumental chemical analysis. **Required fields of study for the professor:** B.S in Chemistry, B.S. in Chemical Pharmaceutic Biology, Chemical Engineering, Specialty or Graduate program in Instrumental Chemical Analysis.
Authors of this learning unit. **Academy:** Analytical instrumentation **Members:** Master María Teresa García Martínez Master Rosalía Palacios Juárez Master Bernardo Gudiño Guzmán Dr. Raquel Treviño Ortiz Dr. María Olivia Peña Ortiz Dr. Gilberto Velázquez Juárez
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