# Test Project

## *Chemical Laboratory Technology*

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# Introduction

The primary objective of this task is to purify citric acid by converting it into an insoluble calcium salt—specifically, tricalcium dicitrate tetrahydrate.

### Chemical Principle of Precipitation:

Citric acid is a weak acid. To induce precipitation, its carboxylic groups must be fully deprotonated into citrate anions, enabling them to react with calcium ions to yield a salt of very low solubility in hot, neutral-to-alkaline aqueous media.

The overall ionic equation for the precipitation reaction is:

$$2C_6H_8O_7 + 3Ca^{2+} + 6OH^- + 4H_2O \rightarrow Ca_3(C_6H_5O_7)_2 \cdot 4H_2O(s) + 6H_2O$$

The presence of $OH^-$ ions in the equation highlights the need for an alkaline medium, either through the choice of a calcium source that simultaneously acts as a base or the use of a neutral source combined with a neutralizing agent.

# Description of project and tasks

This module consists of the extraction, purification, and quantification by UV-VIS spectrophotometry of the citric acid contained in lemon juice.

The steps you must develop are listed below.

- Planning
- Sample preparation, extraction, and regeneration.
- Calibration curves.
- Derivatization and UV-VIS Spectrophotometer quantification.
- Data Processing
- Conclusion.

Each of these items is detailed in the “Instructions to the Competitor” section.

**The duration of this module is 4 hours.**


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# Instructions to the Competitor

In this section, you will be guided on the practical part of the work and on what your report must contain.

## Planning

In your planning, present:

1. A list or table with all reagents that will be used during the task, their respective principal Globally Harmonized System (GHS) code, and your protective actions. You must show this list to the Experts before starting the experimental work.
2. The list of solutions you will prepare – detail the volumes to be prepared and the concentrations. For solutions prepared from solid reagents, consider a tolerance of ± 0.0100 g.
3. Your justified decision on which reagent you chose for the precipitation of tricalcium dicitrate tetrahydrate.
4. The estimated consumption of precipitating agent and sulfuric acid.
5. The detailed procedure from the extract aliquot sampling to the addition of the acetic anhydride and pyridine, that you will use in the derivatization of Citric Acid.
6. The concentration ranges in mg/100 mL chosen for the calibration curves you will prepare.

## Sample preparation, extraction and regeneration

You are receiving a lemon juice sample that has already gone through the following processes (A – Crushing of the lemons, B – Adsorption with PVPP, C – Adsorption with Activated Carbon, and D - Filtration). This sample contains between 3% and 7% (w/v) of citric acid.

Heat 100 mL of the sample to 40 ± 5°C (take the measurement and show it to the Experts) and maintain this heating until the addition of the precipitating agent is complete.

If you deem it necessary, adjust the pH of the sample to 9.00 ± 0.20 using a NaOH solution with a maximum concentration of 3 mol/L.

Add a 10% excess of the chosen precipitating agent and heat the mixture to 90 ± 5°C. Maintain the heating for at least 20 minutes. **Call the experts and show your precipitating agent excess calculations.**

Vacuum filter the formed precipitate and wash it thoroughly with water. Keep the vacuum operating until the filter stops dripping water.

Weigh the solid to estimate the mass of tricalcium dicitrate tetrahydrate. Consider that the moisture content of the solid is 50%.

With your estimate, calculate the necessary amount of sulfuric acid for the regeneration of citric acid. Dilute the calculated Sulfuric Acid in approximately 40 mL of water.

Resuspend the tricalcium dicitrate tetrahydrate in 50 mL of water and add the sulfuric acid solution dropwise until you add 90% of the sulfuric acid planned.

Filter again and wash with small portions of water.


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# Calibration curves

Prepare three (3) calibration curves, with at least five (5) points each, using the citric acid available in the reagent cabinet of this module. The curves will be:

1. Refractive index vs Concentration;
2. Density vs Concentration;
3. Absorbance vs Concentration.

# Derivatization and UV-VIS Spectrophotometer quantification

Use an aliquot of your extract to perform the derivatization followed by spectrophotometric quantification of citric acid. For your extract, carry out this procedure in a triplicate.

Also perform the derivatization of the calibration curve that you will use in the spectrophotometer.

Acetic anhydride reacts immediately with water, forming acetic acid. Other solvents, such as glacial acetic acid, acetone, and ethyl acetate, are suitable for this analysis. Therefore, find a way to replace the water present in the extract with another solvent available in this module's cabinet.

Due to the high apparent molar emissivity of the chromophore that will be formed (approx. 3000 L.mol-1.cm-1), the most concentrated point of your curve must not exceed 10 mg/100 mL.

Add 1 mL of the sample, 1.5 mL of pyridine, and 6 mL of acetic anhydride to test tubes. Shake vigorously after each reagent addition. Pyridine and Acetic Anhydride must be always manipulated at the fume hood. Cuvettes must be covered before being removed from the fume hood.

Place the tubes in a heated water bath at 32 ± 2 °C and maintain for 30 ± 2 minutes. Remove the tubes from the heated water bath and perform a scan between 400 nm and 430 nm (with a 2 nm interval) for one (1) of the points on the curve. Export the scan as an image and add it to your Microsoft Word report.

Select the wavelength you deem most appropriate for your analysis and justify it in your report. **Call the experts and show the wavelength you selected.**

Take the necessary readings and create the calibration curve directly on the spectrophotometer. **Call the Experts to show them the readings and the curve. They will take photos with the tablet.**

Obtain the citric acid concentration in your sample and, from this concentration, calculate the mass of citric acid present in your extract just after the regeneration process.

Export the sample reading results and the calibration curve (with linear equation and $R^2$) using the flash drive and paste them into your Microsoft Word report.

# Quantification by density and refractive index

Use the refractive index vs concentration and density vs concentration curves that you constructed to re-quantify the citric acid in your extract. Present, in your report, the concentration and the total mass of citric acid in your extract.


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## Task questions

1. What is the mass of citric acid present in your extract? Consider the spectrophotometric analysis for this answer.
2. What processes would you apply to the obtained extract to continue the process and obtain high-purity crystals? For this answer, you may consider equipment and reagents that are not available in the Workshop.
3. Compare the concentration of citric acid in the extract obtained by the three (3) methods and comment on the deviations, listing possible contaminants, the limitations of each method, and improvements that could impact results positively. Consider the spectrophotometric analysis as the reference.

## Conclusion

All text (measurement records, discussion, planning justifications, conclusions, answers to the task questions, etc.) must be submitted within the task duration in a text file saved in PDF format. It can be submitted in your native language. **Any other format will not be considered in the assessment.**

## Data processing

The calculations for,

1. Planning
2. Solution preparation
3. Sample preparation
4. Derivatization and UV-VIS quant.
5. Quant. by density and refractive index
6. Task questions

must be **identified and numbered using exactly these headings** and may be submitted either handwritten or in an Excel spreadsheet. For handwritten work, use the standardized sheets provided by the Experts. For Excel spreadsheets, use a separate tab for each of the six (6) headings and submit the .xlsx file within the allotted task time.

# Other

List of annexes:

7. Safety data sheets for all chemical reagents of the Competition, in digital format.
8. Chemical reagents consumption sheet, in digital format.
9. List of protective and emergency equipment available in the workshop and location map.
10. Instructions for waste disposal.
11. Basic infrastructure list for this module.

Check if you have received all these annexes and immediately inform the Experts if any are missing.


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