Part A: Buffer and standard solution preparation

1) Phosphate buffer (PB) 0.1 M pH = 7 preparation

Calculate the quantity of PB (MW = 119.98 g/mol) to be weighted in order to prepare a 100 mL solution of PB 0.1 M. Correct the pH to 7 by using NaOH 5 M. Release of NaOH 'drop-by-drop' till the pH = 7 is reached.

2) Mother solutions of Caffeic Acid (CF) and p-Coumaric Acid (CM) preparation

Calculate the quantity of CF (MW = 180.16 g/mol) and CU (MW = 164.05 g/mol) to be weighted in order to prepare a 5 mL solution at the concentration of 20 mM in methanol for both the compounds.

3) Intermediate mother solutions of CF and CU preparation

Starting from the mother solutions prepared in step 2, prepare intermediate mother solutions in PB 0.1 M pH = 7 (prepared in step 1).

- CF 250 µM
- CU 250 µM
- CF 250 µM e CU 500 µM

Part B: Samples preparation

1) Prepare a solution 80:20 (v/v) MeOH/H_2O
2) Weight 500 mg of sample
3) Add 10 mL of the solution prepared in step 1
4) Orbital shaker agitation for 1 h (in the dark)
5) Filtration with the syringe by using PTFE 0.45 µm

Part C: Samples solution preparation for standard addition calibration

1) Dilute the samples:

- Oregano: 1.10000
- Parsley: 1.1000
- Mint: 1.1000

2) Calculate the volume of standard solutions to be added to each sample.

NB: pay attention to the total volume reached in order to calculate the exact concentration of the analytes!

Oregano

STANDARD ADDITION			Diluted sample	CF + CU	Calculate the final volume
	CF (µM)	CU (µM)	(µL)	(µL)	(µL)
0 (sample)	-	-	1000		
1°	2.5	5	1000		
2°	5	10	1000		
3°	7.5	15	1000		

Parsley

STANDARD ADDITION			Diluted sample	CU	Calculate the final volume
	CF (µM)	CU (µM)	(µL)	(µL)	(µL)
0 (sample)	-	-	1000		
1°	-	2.5	1000		
2°	-	5	1000		
3°	-	7.5	1000		

Mint

STANDARD ADDITION			Diluted sample	CF	Calculate the final volume
	CF (µM)	CU (µM)	(µL)	(µL)	(µL)
0 (sample)	-	-	1000		
1°	2.5	-	1000		
2°	5	-	1000		
3°	7.5	-	1000		

Sage

STANDARD ADDITION			Diluted sample	CF	Calculate the final volume
	CF (µM)	CU (µM)	(µL)	(µL)	(µL)
0 (sample)	-	-	1000		
1°	2.5	-	1000		
2°	5	-	1000		
3°	7.5	-	1000		

Part D: Running DPV measures

Drop 80 µL of the solution onto the electrode surface. Ensure to cover the whole electrode.

Record the peak values (current) obtained for CF and CU measurements.

Parameters

- t equilibration: 5s
- E begin: -0.35 V
- E end: 0.6V
- E step: 0.0025V
- E pulse: 0.05V
- t pulse: 0.05s
- scan rate: 0.025 V/s

Oregano

STANDARD ADDITION			CF-like peak	CU-like peak
	CF (µM)	CU (µM)	(µA)	(µA)
0 (sample)	-	-		
1°	2.5	5		
2°	5	10		
3°	7.5	15		

Parsley

STANDARD ADDITION			CU-like peak
	CF (µM)	CU (µM)	(µA)
0 (sample)	-	-	
1°	-	2.5	
2°	-	5	
3°	-	7.5	

Mint

STANDARD ADDITION			CF-like peak
	CF (µM)	CU (µM)	(µA)
0 (sample)	-	-	
1°	2.5	-	
2°	5	-	
3°	7.5	-	

Sage

STANDARD ADDITION			CF-like peak
	CF (µM)	CU (µM)	(µA)
0 (sample)	-	-	
1°	2.5	-	
2°	5	-	
3°	7.5	-	

Part E: Building of standard addition plot

1) Plot the height of the peaks vs. [µM] of the standards for all three components, including the unfortified sample **(remember to use the final concentration of the standard in the final volume**)

2) Determine the least-squares value of the resulting plots, and record both the slope and intercept.

3) Determine the [µM] of the **CF and CU equivalents** in the unknown sample from the equation: [µM] = -q/m (put y=0). Pay attention to the dilution factors.