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Name Abdul Fayeed	Lab Partner Justin M	Locker/ Desk No.	Course & Section No. 621L

Prelab Questions

1. Chlorogenic acid contains ester bond which is very sensitive to basic solution. It will get hydrolyses, producing caffeic acid and quinic acid. Basic extraction will increase the contamination by caffeic acid and quinic acid. Slightly acidic solution will ensure the caffeine get removed easily.

2. The presence of air bubbles can modify the flow of mobile phase through the column. The pressure in the stream should be maintained all the time.

3. Trifluoroacetic acid is a common acidic HPLC mobilephase modifier, where it acts as ion-pairing reagent. It equilibrates so quickly in the solution that only a small amount is needed for compounds that act as weak acids/bases.

4. Greater flow rate will reduce the retention time, thus causing poor separation. If time were not a factor, it is prefered to have a slower flow for better separation.

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Objective

The purpose of this lab is to analyze the caffeine and chlorogenic acid in green and roast coffee beans by extraction and high pressure liquid chromatography.

Introduction

HPLC is used ~~as~~ for mixtures that contain non-volatile or thermally unstable compounds. Aside from caffeine and chlorogenic acid, there are also presence of contaminants: caffeic acid and quinic acid. Proper choice of ~~C~~Aractor can ensure the best separation. The composition of the caffeine / chlorogenic acid might change before and after ~~the~~ roasting.

Procedure

1. Measure the → check the ref. spectrum refection wavelength for caffeine and chlorogenic acid by using the acetonitrile : water/methanol / mobile phase solution (5% acetonitrile : 95% water/methanol, 80:20 v/v of H_2O:MeOH)
2. Make separate diluted standards for caffeine and chlorogenic acid (stock of caffeine : 0.5mg/mL in solvent, chlorogenic acid : 0.35 mg/mL in solvent).
3. Use 2mL of stock solution and dilute with ~~to~~ the solvent ~~with~~ to 10mL.
4. Add 1 drop of dilute acetic acid to the solution.
5. Record the spectrum of UV-VIS. If A > 1.0, ~~re~~ dilute the solution more, then record the spectrum.

mass of roasted coffee beans

=

mass of green coffee beans

=

$\lambda_{caffein}$ =

$\lambda_{chlorogenic\ acid}$ =

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6. Determine the wavelengths to be used in HPLC.

7. Record the spectrum for both caffein and chlorogenic acid.

8. Choose the λ where caffeine's absorbance is high but low for Chlorogenic acid.

9. Again, choose λ where absorbance of Chlorogenic acid is high but low for caffeine.

10 Make 2 runs on each standard and on each coffee sample : λ from caffeine spectrum, and λ from Chlorogenic acid.

11. Set detection channel @ selected λ, and select 20nm bandwith. Set ref. Chanel to 550 nm, using 100nm bandwith.

12. Prepare 4 standards in 10mL vol. flask Add 0.5, 1, 2, 3 mL of each stock solution (0.5 mL of caffeine + 0.5mL of Chlorogenic acid stock solution) ...

13. Flow rate is set to 1 mL/min. Sampler is set to inject 1 μL sample.

14. Detectable peaks should elute within 5 minutes, But the run will take 15 minutes to ensure No carry-over of undetected compounds.

15. Weigh about 1 g of the ground green and roasted coffee.

16. Place the sample in 250 mL beaker, then add ~ 70 - 75 mL of DI water and clean stir-bar.

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17. Stir slowly and heat the mixture to boiling on the hot plate. Boil gently for about 10 minutes then allow to cool.

18. Filter the cooled mixture through a glass wool plug into a clean 100mL volumetric flask. Rinse the coffee on the filter and add the filtrate into the flask. Dilute with DI water to the mark.

19. Take 2mL of coffee extracts into 10mL vol. flask, then dilute with the solvent.

20. Run the sample unknown sequences after all the standards are done running.

21. Print out & label all 12 chromatographs.

22. Printout & label the UV-vis spectra.

Conclusions

HPLC can be used to analyse the caffeine and chlorogenic acid in green and roasted coffee beans, by using the standard addition method.

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