Chemistry 231 – Spring, 2013
Determination of Trace Phenols in Water by Solid Phase Extraction and Analysis by HPLC
This experiment was contributed by Dr. Forkey and Dr. Baker

Overview

The purpose of this lab is to introduce you to the use of solid phase extraction (SPE) cartridges. Specifically, SPE will be used to concentrate trace amounts of various phenols in water and another sample and to transfer the analytes from water to methanol, which can hold higher concentrations for analysis by HPLC. The performance of the SPE cartridges will be characterized and the trace phenols will be quantified.

Introduction

The extraction of phenols can be important for a number of reasons. Some phenols, like pentachlorophenol and nitrophenols, have industrial uses such in pesticides, anti-fouling paints, and explosive that tend to release them into water sources. This was the focus of past Chem 231 labs. Additionally, lignin, a major polymer in wood, often decomposes (e.g. in firers or through natural aging) to give para-substituted phenols. The main decomposition products tend to be guaiacol compounds, which are para-substituted phenols with a methoxy group ortho to the hydroxy group, and syringol compounds, which are para-substituted phenols with two methoxy groups ortho to the hydroxy group. Separation of guaiacols from corresponding syringols tend to be difficult due to nearly identical polarity. Three of our five phenol standards, 4-hydroxy-3-methoxy cinnamaldehyde, isoeugenol, and vanillin are examples of guaiacols that can be found in foods such as wines aged in toasted oak. In addition, 4-ethyl phenol, also can result from similar lignin sources, but generally is considered to give an undesired odor in wine.

For this experiment, you will be using Varian 'Bond Elut' 200mg C_{18} (octadecyl) SPE cartridges. These cartridges have the same stationary phase as the HPLC columns that we are using for this class. However, the SPE cartridges tend to be less efficient than an HPLC column due to shorter bed length, and larger particle sizes. These features make SPE cartridges relatively inexpensive and disposable.

Liquids may be passed through SPE cartridges either by pulling them through with a vacuum, pushing them through with a syringe, or by pushing them through by placing the SPE cartridge into a centrifuge. For this experiment, you will be using syringes.

Analysis of your phenols, once they are concentrated and in the appropriate solvent, will be carried out by HPLC.

Experiment

You will receive an aqueous solution that contains a trace amount of two phenols. It is your job to determine the identity and concentration of these two compounds. The possible phenol compounds are:

4-ethylphenol
4-hydroxy-3-methoxy cinnamaldehyde
isoeugenol (2-methoxy-4-(prop-1-en-1-yl)phenol)
phenol
vanillin (4-hydroxy-3-methoxybenzaldehyde)

You will be provided with stock solutions (in the 1000 µg/mL range) that can be used to make standards. These stock solutions are made with some amount of methanol (this should be listed on the bottles).

Solid Phase Extraction

SPE cartridges are used in the following order: 1) condition with release solvent, and then with sample solvent, 2) add sample, 3) add sample solvent "wash", and 5) add release solvent. It is also important to not transfer solvents through the column too fast, as this will make phase partitioning inefficient. Flow rates of under 8 mL/min should be used for conditioning and under 4 mL/min for sample transfers and rinsing. The instructions provided are for using syringes to force liquids through the SPE cartridges. However, we may also be able to use a SPE vacuum manifold. The SPE vacuum manifold has the advantage of being able to process numerous samples in parallel. If it is available, the instructor will provide instructions on its use.

To condition the SPE cartridge, before applying your sample, run approximately 20 mL of HPLC grade methanol through the cartridge. It is best to apply liquid to the cartridge reservoir, and then push it through using the syringe set up. Be sure to collect the waste and not to fill the reservoir so high that the stopper contacts the methanol. Then rinse the cartridge with about 10 mL of wash solution. The wash solution for this experiment should be identical to the solvent that contains your unknown (mostly, in this case, HPLC grade water). Make sure that the cartridge does not go dry before applying your sample.

Apply 50.0mL of your sample, in portions, to the SPE cartridge. (It is suggested that you make up a ‘standard’ unknown so that you can practice using the SPE apparatus without using up your unknown solution. You need to do this anyways for the testing of the SPE efficiency.) It is important that you precisely know the volume of sample that you use. The flow rate through the cartridge should be 2 to 4 mL/min. After your sample is applied, wash the cartridge with about 5 mL of wash solution. Again, avoid having the cartridge go dry before or after applying the wash solution. At some point you may want to collect the sample and/or wash solvent to test for "break-through" of the sample analytes.

At this point, you will need a 5 mL volumetric flask to be placed under the SPE cartridge. This can be done by pushing some methanol through with a syringe. Place the cartridge back on the manifold and put a clean 5 mL volumetric flask in place to collect effluent from your cartridge. Draw 5 mL of HPLC grade methanol through the cartridge and into the volumetric flask. Leave the vacuum on until the methanol stops dripping. Remove the volumetric flask and bring the volume up to the mark with HPLC grade methanol. This solution is ready to be analyzed by HPLC.

Identification of Unknown Phenols

Begin by developing a method to separate the five possible phenols listed above in the shortest amount of time possible. This should be done in the same manner as was done in the first lab experiments. One approach is to prepare a five component mixed standard and adjust the eluent until five efficiently separated peaks appear. Then, one at a time, spike the solution with a small amount of the stock solution for each compound. Finally, analyze your extracted unknown phenols to determine what two compounds you have.

Testing SPE Efficiency

It is important that you determine the efficiency of the SPE cartridges under the conditions that you are using. This will give you confidence that the method you develop will be effective in determining the concentrations of phenols in your unknown, or, if needed, account for any losses. The goal for this part of the lab is to determine if any of the phenols break through the SPE cartridge before you have finished adding your sample, and to determine how much methanol must be added to the cartridge to quantitatively elute all of the phenols.

Begin by making up quantitative standard solutions that contain each of the phenols that appear in your unknown. These standards should have concentrations on the order of 5 µg/mL, and be made up in HPLC grade water. You may use the concentrated stock solutions provided to make these standards; the amount of methanol in the standard will be insignificant once diluted with water. Run your standard solution through the SPE cartridge just as you would do with your unknown sample. However, for this part of the experiment, collect the last several milliliters of the aqueous effluent and the wash to test for the presence of your unknown phenols. You may have one mixed standard that contains both your unknown compounds. The next step is to elute the phenols by adding methanol to the cartridge. Add 3 successive 2 mL aliquots of methanol to the cartridge, and collect each aliquot separately in 5 mL volumetric flasks. Bring the volume in the flasks to the mark with HPLC grade methanol.

Quantification of phenols by HPLC

The procedure for quantification of your unknown should similar as for the first HPLC experiment using external standards. The data analysis should also be the same. The external standards should be made up in methanol, and do not need to be run through an SPE cartridge. Be sure to analyze your unknown sample at least twice so you can report the final concentrations and standard deviation in the concentration.

Analysis of Lignin Source Sample by HPLC

Phenols resulting from the decomposition of lignin are present in a variety of possible samples. Two examples where these phenols affect flavor are coffee and wine (particularly those aged in oak). Either sample could be analyzed using SPE concentration followed by HPLC analysis. Alternatively, one could extract phenols from roasted wood chips. You will be expected to choose a lignin source sample, and use SPE to extract/concentrate phenols and then analyze the phenols using the HPLC method developed through comparison to your standards. You should be aware, though, that additional phenols may co-elute with the standard phenols (syringols tend to elute at very similar times to guaiacols with the same substituents). Indicate the estimated concentrations of phenols from the standard list found in your sample.

Report

Your report should be double spaced, with 12 pt Times New Roman font and 1 inch margins. Include your name, a title, a 2-3-sentence abstract, a short introduction, experiment description, results and discussion, and a short conclusion. The introduction should refer to past findings of your lignin source sample. In the experiment description be sure to include (in addition to the operating parameters of the HPLC), a description of the SPE set-up, and the final conditions that were used to prepare your unknown samples. Describe your lignin source sample and how phenols were extracted. The results and discussion should include a discussion of the SPE tests, and include that data either graphically or in a table. Be sure to conform to the ‘guidelines for Chem 231 lab reports’ before handing in the report.