Trace analysis of lignin oxidation products using HPLC-HRMS after accumulation by solid phase extraction

Trace analysis methods play a major role in environmental analysis. In addition to the actual analysis with HPLC-MS, for example, this also includes sample preparation. Extraction methods such as solid phase extraction are used for this purpose.

After cellulose, lignin is the most frequently occurring biopolymer and the composition of the monomers can be used to draw conclusions about the type of vegetation. In this experiment, you will obtain an aqueous solution containing very low concentrations of three different lignin oxidation products or other relevant environmental markers. These include, for example, vanillic acid or cinnamic acid. The aim of the experiment is to enrich the compounds using suitable solid phase extraction and then analyse them using HPLC-HRMS.

Group	**Analyts**
1	p-Hydroxyacetophenone Vanillic acid Pinic acid
2	Syringaldehyde Coumaric acid Methanesulfonic acid
3	Ferulic acid Acetovanillone Pinonic acid
4	Salicylic acid Pinic acid p-Hydroxyacetophenone
5	Vanillin Acetosyringone Camphoric acid

Implementation and evaluation

Before day 1 and for the colloquium

Select a suitable material from the following solid phase materials to enrich the compounds assigned to you. Research suitable methods on the manufacturers' websites. Which solvents do you need for conditioning your material and which for elution? Does your sample require a specific pH value?

Available solid phase materials:

Hydrophilic-lipophilic balanced (HLB), 60 mg, Oasis Waters

Strong anion-exchange (MAX), 30 mg, Oasis Waters

Weak-anion exchange (WAX), 60 mg, Oasis Waters

Strong cation exchange (SCX), 1 g, Supelco

C_{18} – Material (DSC-18), 100 mg, Supelco

- Calculate the monoisotopic mass of the deprotonated form [M-H]- of your three analytes to the fourth decimal place.
- Find out about the validation of analytical methods, what parameters are there and how are they determined and calculated? (You can find a ‘Guideline for method validation’ online from the German Federal Environment Agency)
- Think of 7 sensible standard concentrations for a calibration in the range 1-1000 ppb and calculate the volumes to be pipetted based on a stock solution of 10 µg/mL.
- What elution order do you expect for your three compounds and why?
- Prepare carefully for all instruments and techniques used!

Day 1

You will receive a 50 mL flask with a solution containing unknown concentrations of three compounds. Fill the flask with ultrapure water (Milli-Q) up to the calibration mark and take three aliquots of 10 mL each. Spike one of the three samples with 100 ng of your compounds from a stock solution of 10 µg/mL BEFORE the solid phase extraction (you will receive this from the assistants).

Now use the previously selected solid phase material for enrichment. After eluting your samples, add the same amount of standard solution to one of the non-spiked samples as to

the previously spiked sample. You will need the spiked samples later to validate your method for determining recovery and matrix effects.

Evaporate all elutions under a stream of nitrogen at 30°C until dry. Then add 500 µL of an acetonitrile/water (1:9) solution and store your samples in the refrigerator.

To quantify your samples later, prepare a standard series with different concentrations in the range 1-1000 ppb. Select 7 sensible concentrations and prepare them in 1 mL acetonitrile/water (1:9). You will receive a stock solution with a concentration of 10 µg/mL. You will also need a blank sample of 1 mL acetonitrile/water (1:9).

Day 2

Use one of your highly concentrated standard solutions to develop a separation method for your three compounds. The aim is to develop a gradient with which all three compounds are baseline-separated within a maximum of 15 minutes.

A pentafluorophenyl column (PFP) and the following solvents are available for method development

Eluent A: H_2O/ACN (98:2) with 400 µL/L formic acid

Eluent B: H_2O/ACN (2:98)

Using the method you have developed, measure your standard series in duplicate overnight and measure your samples in triplicate. Select one of the standard solutions and measure it five times to determine the repeatability. Measure your blank sample ten times.

Day 3

create a processing method for the automated evaluation of your measurements with the help of the assistant.

With the help of your calibration, you will be able to determine the content of the three compounds in your unspiked sample (note the enrichment factor and enter the error). Determine the following method validation parameters: repeatability, recovery, matrix effects, detection limit and quantification limit. Discuss your method development and your results in your protocol and answer the questions and tasks listed below.

Notes on the determination of method validation parameters:

1. Repeatability

In this test, we only determine the measurement precision within a daily series.

2. Recovery

In this experiment, the recovery of the sample doped before the SPE is to be compared with the recovery of the sample doped after the SPE. Use the following formula for the calculation:

Recovery rate $W = \frac{S_1 - S_2}{S_3} \cdot 100$ [%]

Where S_1 is the concentration of the sample spiked before or after SPE. S_2 is the unspiked sample. S_3 is the concentration of a standard solution, which corresponds to the target final concentration of your spiking. Calculate this and note that you also need this concentration when preparing your calibration solutions.

Notes on the evaluation in general:

Please note that you must first subtract the determined blank value (average value of the 10 measurements of your blank sample) from all values that you use. The blank value cannot be subtracted when determining the detection and quantification limit.

Questions and tasks for the protocol:

- - Justify your choice of solid phase material and separation column.
- - What is the purpose of solid phase extraction apart from enrichment?
- - Why is a prior chromatographic separation useful even when using a high-resolution mass spectrometer?
- - What are the advantages of high-resolution mass spectrometry compared to conventional mass spectrometers?
- - Explain the relevance of your analytes in the environment.