Analysis of flower volatiles (or cinnamaldehyde in cinnamon sample) by Solid Phase Micro Extraction and Gas Chromatography

OVERVIEW

The purpose of this experiment is to introduce to the use of solid phase microextraction (SPME). In this experiment the SPME will be used to concentrate flower volatiles from a flower (spring/summer period) or cinnamaldehyde in cinnamon sample (autum/winter period). Analysis will be accomplished by using gas chromatography (GC) with MS detector.

INTRODUCTION

In analysis of volatile and semi-volatile flavor or fragrance components, such as the flower specimen or cinnamon sample in this experiment, a preliminary cleanup step is often needed before the chromatographic separation (e.g., extraction, preconcentration, removal of impurities). Traditional methods of steam distillation, solvent extraction, purge-and-trap or various other techniques typically require excessive time, complicated equipment, and/or use of excess of organic solvents.

Solid-phase microextraction (SPME) is an interesting and promising technique for the extraction and concentration or enrichment of volatile compounds from different sample matrices. The key component is a fiber coated with a polymer and/or a solid sorbent. In the commercial syringe–like holder, the fiber is attached to a fixed metal needle in the base. The fiber can be extended from the needle or retracted inside the needle. The extended fiber is either immersed in the sample solution (direct immersion, DI-SPME), or exposed to the vapor above the sample (headspace, HS-SPME). For this experiment, a Supelco polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65µm SPME fiber will be used. In our experiment sampling the gas phase will be made. In general, after a suitable extraction time, the fiber is retracted, and the needle is inserted into the injection port of a GC (or an HPLC using a special interface). The extracted components are desorbed and subsequently separated. For analyses of food aroma compounds, the GC effluent is usually detected by mass spectrometry. In SPME, the partition coefficient is dependent on both the properties of the fiber and the analyte. The amount of analyte partitioned by the fiber is proportional to the initial concentration of the compound in the sample. Complete extractions are usually not necessary to obtain quantitative information, since the amounts of the analytes in the SPME phase are controlled by the various distribution constants in effect under the experimental conditions. As long as the conditions are carefully repeated from run to run, the ratio of concentrations in the phases also will remain constant.

With favorable conditions, the mass spectrum shows the peak of the molecular ion and characteristic signals corresponding to the individual fragments. The task of identifying an unknown compound is greatly simplified by comparing the mass spectrum with spectra stored in a reference library. With suitable calibration, usually by the internal standardization method, MS is also a very sensitive tool for quantitative analysis.

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EXPERIMENT

Identification and quantification of typical flower volatiles

You will receive a solution that contains two known flower volatiles. The compounds will be dissolved in hexane/acetone. It is your job to determine the identity and concentration of these two compounds by GC. The possible volatiles are:

- geraniol
- linalool
- limonene

You will be provided with stock solutions (in the 2000 µg/mL range) that can be used to make standards. These stock solutions are made with hexane/acetone.

Solid Phase Microextraction

Place a fixed volume of above sample into a vial that has a cap with a septum. Because it is difficult to consistently collect the same amount of sample each time you use the SPME device, you should include an internal standard with all of your solutions.

Gas Chromatograph

For this experiment you will be using a Varian GC with a mass detector. The instructor will give you a quick run-down of this instrument before you get started.

Analysis of Unknowns

Be sure to analyze your unknown solution at least three times, and more if time permits so that you may report uncertainty.

Quantification by GC

You must use an internal standard for this lab. Be sure to choose a compound as your standard that can easily be separated from the analytes in your sample, and is completely soluble in the solvent you are using. The following lists the steps necessary to use an internal standard for quantitative analysis.

1. Let X = the unknown, and S = the standard.

$$\frac{A_X}{A_S} = F\frac{c_X}{C_S}$$

where A is the area, C is the concentration, and *F* is a 'response factor' C_S between X and S.

1. A solution is made that contains known amounts of X and S, this solution is used to determine *F*.
2. Add a known amount of S to any solution that contains an unknown amount of X.
3. Realize that this will dilute the solution, and this must be accounted for when calculating the final concentration of X.
4. After obtaining a chromatogram of the solution, use the equation in '2' above, to determine C_X. Be sure to account for the dilution that occurred when adding a known amount of S.

Flower or cinnamon volatiles

After you have identified and quantified the compounds in your sample, you will analyze some compounds from actual flowers or cinnamon. Bring in several fresh flowers (of any type, but all of the same type, and know the name of the flower) for analysis. You should try to analyze the volatiles from at least: the petals, stamens, and pollen.

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Place the flower parts into a vial with septum. Let the samples sit for at least 30 minutes so that the volatiles have a chance to diffuse from the flower parts. Be sure that the SPME fiber will not touch any of the flower parts during sampling. Analyze the sample just as you did the samples derived from solution. Since it is not possible to use an internal standard, we are not concerned with quantifying the absolute amount of each compound emitted by the flower parts. Instead, make an evaluation of the relative amounts of each compound. If a compound cannot be identified, simply identify it by number, and only be concerned with compounds that make up more than 5% of total emissions.

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