Gas Chromatography II. Quantitative Gas Chromatography

Purpose

In this experiment you will use the internal standard technique for quantitative analysis of a mixture of normal alkanes by open-tubular gas chromatography using splitless injection and temperature programming.

References and Apparatus

Please refer the the previous GC experiment, "Gas Chromatography I. Retention and Efficiency."

Apparatus

An Agilent 6850 GC with a HP-1 analytical capillary column and thermal conductivity detector (TCD). ChemStation Software. Please refer to the experiment "Gas Chromatography I. Retention and Efficiency" for details.

System configuration

Injection mode: Splitless
Column: HP-1 capillary column (isothermal Tmax = 325 ^{o}C); 30 m long x 0.32 mm ID x 0.25 μm film thickness. 100% methyl-substituted polysiloxane (dimethylpolysiloxane).
Detector: A modulated single-filament thermal conductivity detector (TCD).

Chemicals:

Pure solvents and standards

Iso-octane (2,2,4-trimethylpentane)
1, 2, 3, 4-tetrahydronaphthalene (tetralin)
Mixed n-alkane standard ($C_{11}H_{24}$, $C_{12}H_{26}$, $C_{13}H_{28}$, $C_{14}H_{30}$), equal masses, neat.

Prepared standards

- 1, 2, 3, 4-Tetrahydronaphthalene (tetralin), 1.0 μL/mL in isooctane, or 0.1 % (v/v).
- Mixed standard containing C11, C12, C13, C14, 0.125 % (v/v), each solute, in isooctane. Prepared by adding 50 μL of the neat mixed standard to isooctane and diluting to exactly 10 mL.

Theory

Retention and efficiency in gas chromatography were addressed in a previous experiment. In this experiment we turn our attention to the use of gas chromatography for quantitative analysis.

Quantitative analysis of mixtures by GC requires

- qualitative identification of individual peaks
- quantitative measurement of peak area and its relationship to quantity of solute

Qualitative identification of peaks is generally accomplished by matching the retention time of an analyte peak with that of a standard obtained under identical conditions. In complex mixtures, or those containing solutes that elute with similar retention times, the use of an information-rich detector, such as a mass spectrometer, is highly desirable. Such a detector has the ability to distinguish between two or more solutes eluting together in a single peak, and also provides mass spectral information for qualitative identification. For simple mixtures where overlapping peaks are not a problem, a simple detector such as the thermal conductivity detector or flame ionization detector is satisfactory. In such cases the analyst must rely on general knowledge of the sample composition and possible interferences, and in any case should closely examine the chromatographic peaks for any abnormalities, such as a shoulder or other distortion of the peak, that might be due to a coeluting interference.

Quantitative analysis is generally based on measurement of peak area. Although peak heights can be measured more easily, they are more sensitive to minor changes in conditions and injection technique. Modern chromatography data systems include data acquisition and analysis software that simplifies the measurement of peak areas, and these days essentially all peak quantitation is based on the measurement of peak areas. Depending on the complexity of the mixture and the chromatographic technique used, there are several approaches to using peak areas for quantitative analysis.

External Standard Method

A calibration curve can be constructed by separate injections of standards. The unknown is then injected and the signal is compared to the standard curve to determine the concentration or amount of analyte. Although simple in concept and approach, this method suffers from poor reproducibility due to the difficulty of reproducing small injection volumes, especially when using manual syringe injection.

Internal Standard Method

In this approach, an internal standard is added to the sample, and the response from the analyte peak is compared to the internal standard. To compute the amount of analyte, a response factor is applied that accounts for the differences in detector response for the two compounds. This approach corrects for variations in the injection volume.

The response factor is measured by injecting a mixture containing known amounts of analyte and standard. For some analyte X, the response factor is

$$R_{X/IS} = (A_X/A_{IS})(m_{IS}/m_X)$$

where

A_X, A_{IS} are the peak areas for the analyte and internal standard
m_X, m_{IS} are the masses of analyte and tetralin internal standard injected

In the analysis of an unknown sample, a known amount of internal standard is added to the sample prior to injection. The amount of unknown X in the sample is determined simply by solving the above equation for m_X.

Experiments:

General Operating Conditions.

Instrument Settings

For all of the experiments described below, use the following configuration. These settings may be already be saved as a method which you can load (see instructor).

Inlet: Splitless.
Column head pressure: psig He
Inlet temperature: 250 ^oC
Detector temperature: 350 ^oC
Temperature program:
  Initial temp: 50 ^oC, 2 minute hold time
  Ramp 1: 12.0 ^oC min^{-1} to 120 ^oC, 0.10 min.
  Ramp 2: 30.0 ^oC min^{-1} to 280 ^oC, 2 min.

You may need to adjust these settings to obtain acceptable data, but do not exceed 325 ^oC for the column oven, as this may permanently damage the column. Record all operating conditions for every run you perform. Print the method parameter sheet for each method used, and print a copy of each chromatogram.

Injection Procedure

Injection volume: 1µL.
For all injections, fill the syringe and adjust the volume to 1 µL of sample. Perform the injection in a smooth, rapid, continuous motion as demonstrated by the instructor.

A. Determination of Response Factors

Inject 1 µL of the 0.1 % tetralin standard to determine its retention time. Repeat for the 0.5 % mixed n-alkane standard.

Prepare a series of three standard solutions containing the same amount of internal standard tetralin and varying amounts of the mixed n-alkane standard, by adding the following amounts of the indicated solutes to a series of 10 mL volumetric flasks and diluting to volume with isooctane.

Standard	Tetralin µL	n-Alkane Mixed Std µL	Vol % Alkane Std Total	Vol % Alkane Std Each alkane
1	10	200	2.0	0.50
2	10	50	0.5	0.125
3	10	10	0.1	0.025

Inject 1.0 μL of each prepared standard in triplicate. For each of the four n-alkanes, prepare a plot of A_X/A_{IS} *vs* m_X/m_{IS}. In each case you should obtain a straight line with slope equal to the response factor for that solute.

B. Analysis of an Unknown Mixture

You will determine the mass percent of each *n*-alkane in one or more old samples of the mixed standard. Prepare each sample as described for standard #2 above. That is, add 10 μL of tetralin and 50 μL of the neat sample to a 10 mL volumetric flask and dilute to volume with isooctane. Make three successive injections of each of the prepared samples and determined the mass percent of each of the *n*-alkane solutes present in each.

C. Detection Limits

The detection limit for a GC analysis can be estimated by comparing the signal for a standard near the detection limit to the noise in the baseline. At the 95 % confidence level, the signal corresponding to the minimum detectable amount of sample is

$$S_{LOD} = 3\sigma_b$$

where σ_b is the standard deviation in the baseline signal. The latter can be shown to be approximately equal to one-fifth of the peak-to-peak noise in the baseline. Using this criterion, report the detection limit (in μg) for each of the n-alkane solutes.