Analysis of Pesticides and Herbicides by Gas Chromatography/Mass Spectrometry
Instrumental Methods for Environmental Analysis
CHEM 467

Sample Preparation

Samples are prepared for analysis using modified standard EPA protocols for primary pollutant analysis. Weigh out approximately 2 – 3 g of each type of food to be analyzed and record the mass of each sample. Macerate each sample with a 25 mL aliquot of pesticide residue analysis (PRA) acetone, followed by addition of a 25 mL aliquot of 2% sodium hydroxide (NaOH). Blend samples for 2 – 3 minutes or until homogenized. Add a 25 mL aliquot of PRA hexane and mix. Transfer the multiphase mixture to a polypropylene centrifuge tube and centrifuge (1000 X g) for 5 minutes to separate the nonpolar fraction of the mixture (top layer) from the polar aqueous fraction (lower layer). Transfer the upper hexane fraction to a 250 mL Erlenmeyer flask using disposable pipettes. The lower layer is returned to the blender and the hexane extraction process is repeated twice more to give a total volume of 75 mL hexane extract. Combine the hexane fractions and make up to volume in a 100 mL volumetric flask.

Analysis of pesticides by gas chromatography/mass spectrometry

Gas chromatography introduction

Reference: Chapters 20, 26, and 27—*Principles of Instrumental Analysis, 5th Edition* Skoog, Holler, and Nieman, Saunders College Publishing

Chromatography is a method used in the separation of mixtures. The theory behind chromatography is reviewed in chapter 17 of the above reference and will only be summarized here. The efficiency with which a chromatographic column separates a mixture into its individual components is expressed as the effective number of plates of the column. This terminology is related to the number of plates in a distillation column. The number of effective plates is determined from the elution of a single component and is defined as

$$N_{eff} = 16(t'_R/W_b)^2$$

where t'_R is the time required for the band center to elute, and W_b is the width of the eluted band where the width is determined by extending lines tangent to the inflection points on the side of the peak to the baseline and measuring the distance in time between the intersections of the tangent lines and the baseline. This formula assumes that the peak is Gaussian in shape. Another related expression for the number of effective plates is

$$N_{eff} = 5.54(t'_R/W_{1/2})^2$$

where $W_{1/2}$ is the peak width at the half maximum. This form is less sensitive to baseline drifts and peak asymmetries which often occur.

Another parameter of importance is the height equivalent to an effective plate or H. H is the spacing between the effective plates. It can be measured empirically from the length of the column and the number of effective plates:

$$H = L/N_{eff}$$

where L is the length of the column. Theoretically, H is represented by the van Deemter equation:

$$H = A + B/\mu + C_{stationary}\mu + C_{mobile}\mu$$

where μ is the linear velocity of the mobile phase (carrier gas), A is a constant related to eddy diffusion or the tendency of the gas to move perpendicular to the direction of flow, B is a constant related to longitudinal diffusion or the tendency of the gas to move parallel to the direction of flow, and the constants $C_{stationary}$ and C_{mobile} are related to the resistance to mass transfer at the solute/stationary phase interface. The flow rate through the column is most efficient when H is at its minimum.

The final parameter of interest is the resolution of the column. Resolution is the ability of the column to resolve two compounds into separate, distinct peaks. Resolution is defined as

$$R = 2d/(w_A+w_B)$$

where d is the distance between the peaks, and w_i is the width of peaks A and B, respectively.

Ideal resolution for two compounds occurs when R = 1—a value less than one indicates the compounds are not completely resolved, and a value greater than one indicates that the carrier gas flow rate is too slow and time is being wasted.

Analysis by GC/MS

The instructors will explain operation of the GC/MS instrument and assist in analysis of your samples. Standard solutions containing target compounds will be provided by the instructor.

Prepare a calibration curve for each of the target compounds from the integrated peak areas of the standard solutions. From the slope and y-intercept of each calibration curve, determine concentrations (this will be in units of $\mu g\ L^{-1}$) of the target compounds for each sample. The actual concentration in the food samples is calculated by:

$$[X]_{sample}\ (ppm) = \frac{[X]_{soln}(\mu g\ L^{-1}) \cdot 0.100\ L}{m_{sample}(g)}$$

where $[X]_{soln}$ is the concentration determined from the calibration curve, and $[X]_{sample}$ is concentration in part-per-million (ppm) in the original sample. Report sample concentrations with appropriate error bounds for each compound.