An Overview of SW-846 Method 8261 Chemistry*

Michael H. Hiatt
U.S. Environmental Protection Agency
National Exposure Research Laboratory
Environmental Sciences Division
P.O. Box 93478, Las Vegas, Nevada 89193-3478

*Volatile Organic Compounds by Vacuum Distillation in Combination with Gas Chromatography/Mass Spectrometry (VD/GC/MS)

Method 8261

- Method 8261 is a RCRA analytical protocol to determine the concentration of volatile compounds in a variety of matrices.
- The protocol can be applied to many programmatic matrices not amenable to other methods such as oils and biota.
- The method incorporates the physical chemistry of compounds in measuring matrix effects. The accurate assessment of matrix effects yields method performance data by analyte and is the foundation for the method's reporting of results with confidence intervals.
- The discussion that follows explains why the physical chemistry of analytes is so important to the method.

Chemistry Behind Method 8261

- Method 8261 addresses compounds that can be made gaseous in vacuum and which can be separated from a sample matrix.
- The vapor pressure of a compound indicates if it can be made gaseous.
- The degree to which a compound dissolves in and has an affinity for sample matrix describes the difficulty in separating the compound from that matrix. Method 8261 uses the term, “relative volatility”, to describe the degree of volatilizing a compound from water.
- Diffusion of compounds through a sample matrix is not an important variable to address when the matrix is water. Note: Diffusion can be critical when a sample matrix impedes movement of compounds. This will be discussed.

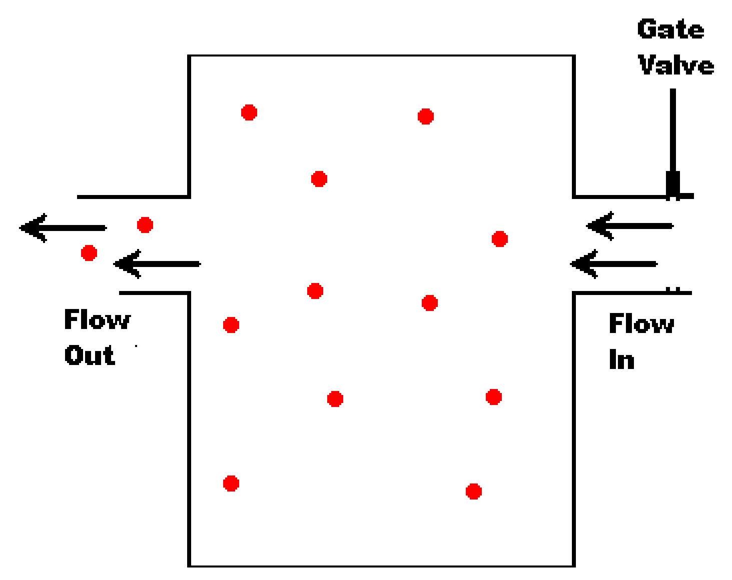
Major Points of Discussion

- Compounds as gases can flow from a container for isolation.
- Getting the compounds into gas form can be inhibited by a sample matrix.
- Once compounds are volatilized and separated from the sample matrix their recovery may be diminished by condensation in the apparatus.
- Internal standards are used to measure the behavior of compounds throughout the analysis.

Compounds as Gas

- Some compounds are gases at room temperature.
- These compounds can be purged from their container by flushing with a stream of gas or pulling vacuum.
- Transfer of these gases by vacuum distillation is used for the detection and measurement by instrumentation such as GC/MS.

Gases are Mobile and Easily Removed from a Container



- A gas flow can flush gaseous compounds from the above container.
- With the gate valve closed, a vacuum at the outflow side pulls the compounds from the container.

Only the Gas Fraction of Compounds are Readily Moved from the Container

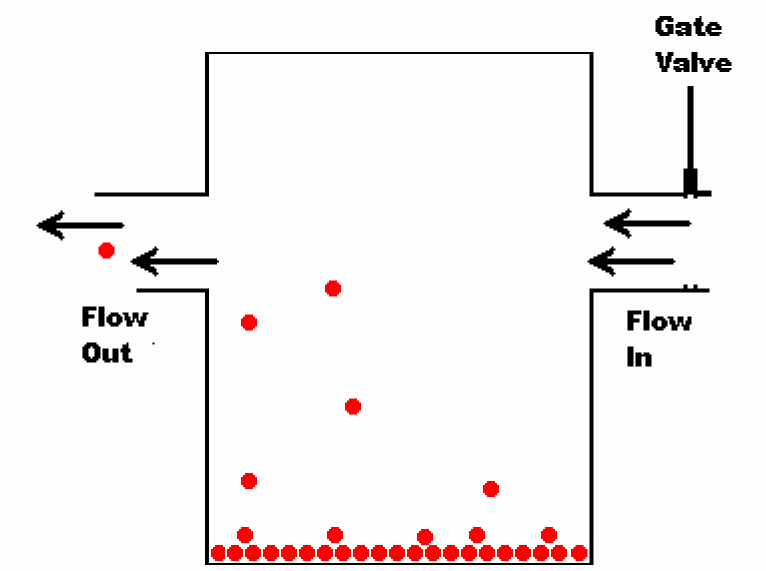
- In the previous slide, gaseous compounds were purged from a container.
- However, some compounds are not entirely gases but are liquids or solids with only a small fraction as a gas.
- The degree to which a compound is a gas is described by its vapor pressure at the surrounding temperature and pressure.

Vapor Pressure

- Liquid or solid compounds may be slowly evaporated from a surface (sample or container) even when conditions are not sufficient to make them gases. These compounds still exert a vapor pressure though much less than the pressure of the system.
- The greater the vapor pressure of a compound at room temperature, the greater percentage of it will be gaseous.
- The vapor pressure of a compound is a function of its boiling point[1].

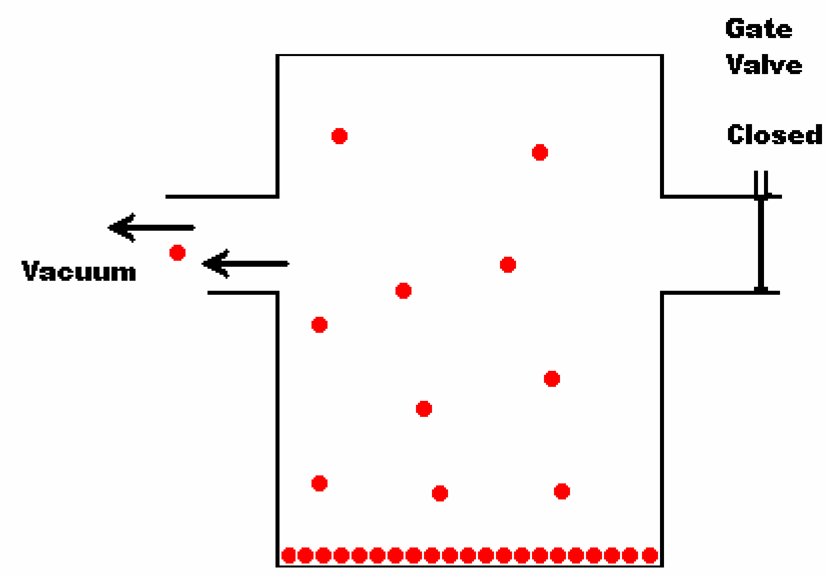
[1] Antoine, C., “Tensions des Vapeurs: Nouvelle Relation Entre les Tensions et les Temperatures, Compt. Rend. 107, 681 (1888)

Liquid or Solid Compounds Are Less Effectively Flushed from a Container



A gas flow can only flush the gaseous fraction of compounds. The gaseous fraction is relative to its vapor pressure at the temperature of the container.

Liquid or Solid Compounds Are also Less Effectively <u>Evacuated</u> from a Container



The introduction of a vacuum source only evacuates the gaseous fraction of compound from the container. However, the vacuum also reduces the pressure in the container, and thus, increases the gaseous fraction of the compound.

Gases – in Conclusion

- Compounds in a vapor phase can be moved from a container with either pulling a vacuum or flushing with gas.
- The fraction of a compound that is present in the vapor phase depends on its boiling point.

What if a Compound is Dissolved in a Sample?

- Introducing a sample matrix introduces a *compartment* that can have a strong attraction for compounds. For ease of discussion, the sample matrix being discussed will be water.
- This new compartment adds an attraction that must be overcome for a compound to be in the vapor phase.
- The tendency of a compound to separate from water and become part of the gas phase above the water can be described as water-to-air partitioning.

Compounds Partitioning between Water and Air

- Just how a compound is distributed between the water and the air is directly related to the compound’s affinity for the water and its being a gas. This is the water-to-air partition coefficient, K_{wa}, and equals concentration of compound in water compared to its concentration in air. In this presentation where water is the given matrix, we’ll just use K.
- The greater the K value, the more a compound tends to stay dissolved in water than in air.

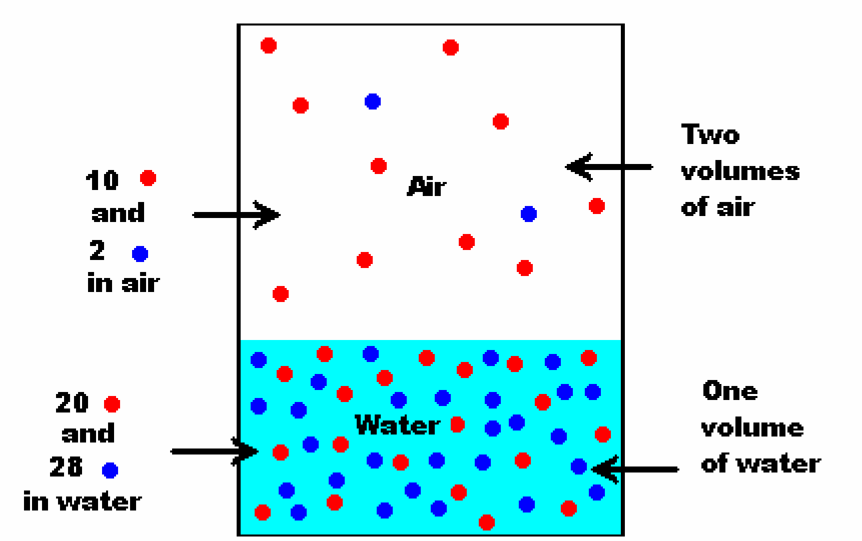
More about Partition Coefficients

- **Partition coefficients describe a steady-state equilibrium condition.**
- **The partition coefficient value is dimensionless as the ratio of the concentration cancels out units.**
- **The value of K is affected by temperature and therefore partition coefficients are for a specific temperature.**

Partitioning Illustration

Assume a 3-volume closed container with two volumes of air and one of water. There are 20 red and 28 blue spheres in the water and 10 red and 2 blue spheres in the air.

What is K for the red and blue spheres?



The concentration of red spheres in water is 20 spheres/volume. The concentration of red spheres in air is 5 spheres/volume (10 spheres in two volumes in the illustration). Therefore for the red spheres, the water-to-air partition coefficient, K is 4. (20 spheres/volume ÷ 5 spheres/volume = 4). For the blue spheres K = 28 (28÷1)

Dynamic Partitioning as Relative Volatility

- While K describes a steady state at equilibrium, flushing or evacuating a container over a time interval is *dynamic* and does not reflect an equilibration.
- Vacuum distillation is a dynamic process where K is used to describe the relative ability of a compound to volatilize.
- The distillation of a compound still relates to its K but in a dynamic way. Chemical engineers use the term *relative volatility* to describe this dynamic partitioning during a distillation. This term describes the *relative* distillation of one compound to another compound as a ratio of their respective partition coefficients. For instance in our previous example of red and blue spheres, the relative volatility of red to blue would be 4 ÷ 28 which equals 0.14.

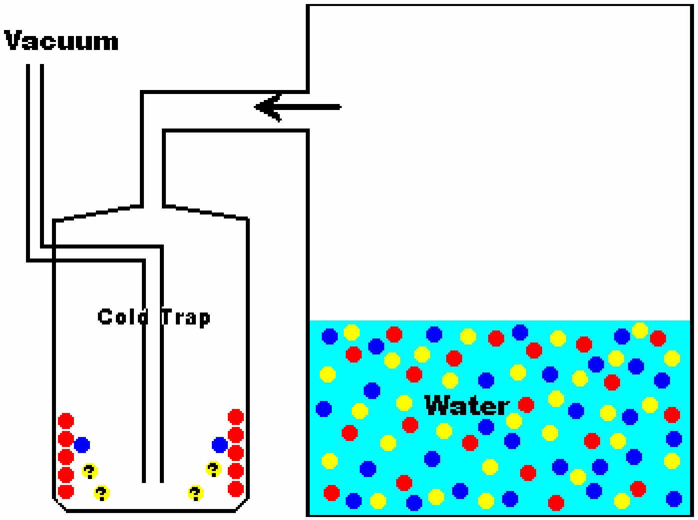
Method 8261's Relative Volatility

- The chemical engineering use of relative volatility is a comparison of partition coefficients and not a fixed value. This is an awkward value when working with many compounds as does method 8261.
- In method 8261, relative volatility values are made to correspond to a compound's partition coefficient. These values are assigned and do not vary by distillation.
- More discussion is available in the publications section of this webpage.[2]

[2] Hiatt, M.H., Farr, C.M, "Volatile Organic Compound Determinations Using Surrogate-Based Correction for Method and Matrix Effects", **Analytical Chemistry,** Vol. 67, No. 2, p. 426-433, January 15, 1995

Relative Volatility Illustration

This time the 3-volume container from the previous partitioning illustration has a vacuum pump attached. A cold trap is added between the container and the vacuum pump. There were 30 red, blue and yellow spheres added to the water. A vacuum is applied for a short period of time. After the evacuation stopped, we find 10 red spheres and 2 blue spheres isolated in the cold trap. Later we will calculate how many yellow spheres also migrated.



- The partition coefficients for the red and blue spheres were previously determined to be 4 and 28, respectively, and we use these values as their relative volatility values.
- Knowing the relative volatility values and the fractions of red and blue spheres collected in the cold trap provides us with the information needed to quantify the migration of spheres from the water to the vapor phase.

Headspace-Equivalent Volume

- The first headspace volume that is removed from a container can be measured as the evacuation of the air from the container.
- The first headspace, however, does not describe the volume removed from a container if we recovery more of a compound than could be in the initial headspace.
- After the air has been removed, measurement of the headspace volume is done by measuring the removal of compounds from the water. That is, if 10 % of a compound is in the headspace before evacuation, an additional "headspace-equivalent" volume must be removed from the container to volatilize 10% of the remaining compound from the water.
- A vacuum distillation can be thought as pulling a series of headspace-equivalent volumes from a container. The total volume H, is the volume pumped through the vacuum during the distillation time.

The Vacuum Distillation Volume, H

- The vacuum distillation volume, H, is important because with it we can interpret the vacuum distillation as creating a large headspace and compounds are distributed in this headspace and a water sample as K (or relative volatility). And as H passes through the cold trap, all the compounds present in the headspace are trapped in a cold trap. The mass of a compound in the cold trap (M_H) divided by H and compared to the mass of the compound remaining in the water (M_w) divided by water volume (V_w) equals K.

$$K = \frac{M_W \div V_W}{M_H \div H}$$

- The above equation can also be used to solve for H if we have added a known amount of a compound (with K), to the water before performing a vacuum distillation. Then by measuring the mass of the compound in the cold trap we get

$$H = \frac{M_H \times V_W \times K}{\text{Mass added to water} - M_H}$$

- Measuring the response of a reference compound, the red spheres in the previous relative volatility illustration, H can be determined. Therefore

  H = K red spheres in trap ÷ concentration in water

  $H = 4 \times 10 \div 20 = 2$ volumes

Using H to Determine Mass Vacuum Distilled

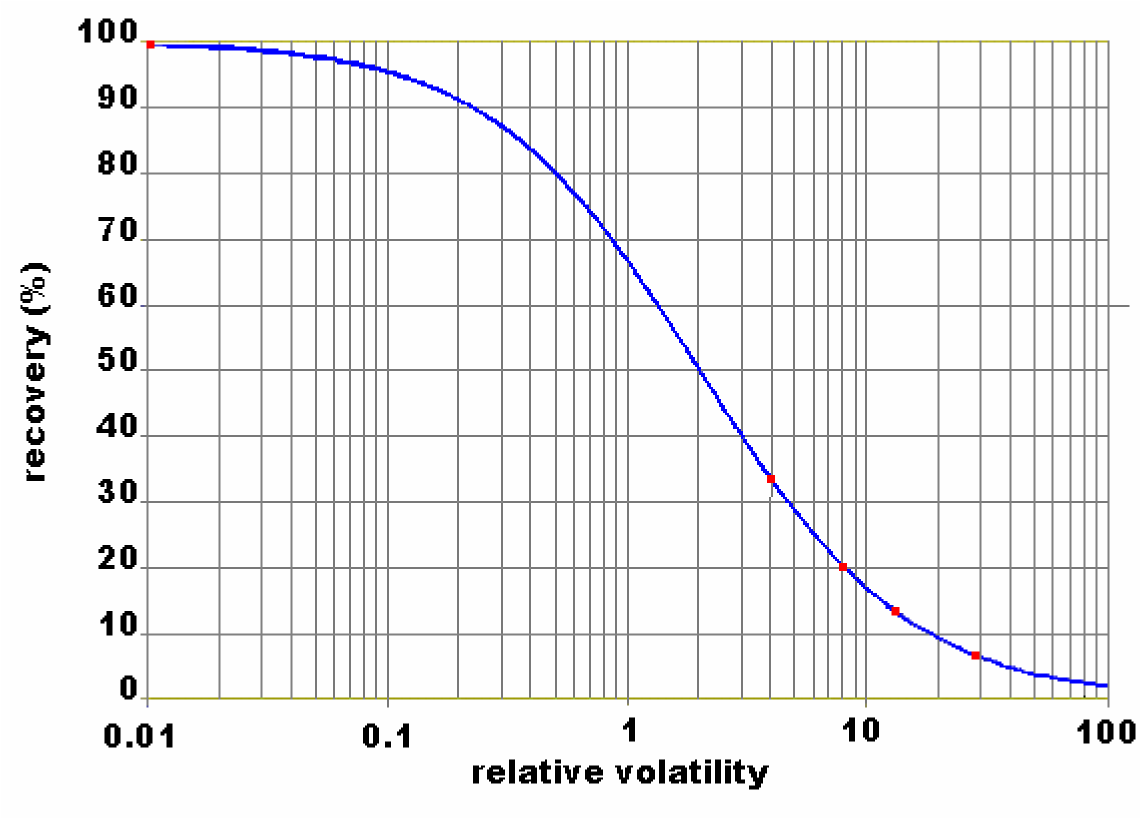
- After H is determined for a vacuum distillation, we can also predict how many of another color sphere would be in the cold trap by knowing its K and concentration in the water before distillation. If the K for yellow spheres in the relative volatility illustration is 8, then there will be 6 spheres in the cold trap ($K = 8 = (30 - x) \div (x \div H)$)
- If a green sphere has $K = 13$, the solution for how many spheres are in the cold trap is 4. If K is 0, all spheres would be in the cold trap and if $K = \infty$, there would be no spheres in the cold trap.

Relative Volatility vs. Recovery of the Example Data

- We can look at the number of spheres in the cold trap and determine the % recovery of spheres. For instance, the recovery of blue spheres is 33.3% (10/30).
- Converting the number of spheres to percentages

  K = 0; Recovery = 100 %
  K = 4; Recovery = 33.3 %
  K = 8; Recovery = 20 %
  K = 13; Recovery = 13.3 %
  K = 28; Recovery = 6.67 %
  K = ∞; Recovery = 0 %
- The plot of recovery vs. relative volatility is on the next slide.

Recovery vs. Relative Volatility for Illustration



Predicting Recoveries

- The use of spheres in the previous slides was a simplification for mass of a compound. From this point on, it is easier to think in terms of recovery of compounds as the fraction of a compound that has been volatilized from the sample.
- It was shown that recovery of an analyte (as mass) during a distillation depends on the relative volatility of the compound. If we added internal standards to every sample of water before distillation, by measuring their recovery we could plot recovery as a function of relative volatility and interpolate the recovery of any other compound if we knew their relative volatility values.
- If we have calibrated the GC/MS, a response of compound A can be translated into mass, M. Interpolating the recovery from the recovery vs. relative volatility generated by the internal standard recoveries, it is easy to identify how much compound A is in the sample

Compound A in sample = M ÷ recovery from plot

Determination of Experimental Relative Volatility Values

The previous recovery vs. relative volatility plot can also be used to experimentally measure relative volatilities. The following process can be used to experimentally derive relative volatility values.

1. Compounds of unknown relative volatilities are added to a water sample containing internal standards of known relative volatility values.
2. The water sample is vacuum distilled and all recoveries of all the compounds and internal standards are measured.
3. A plot of recovery vs. relative volatility is drawn using the internal standard recoveries and relative volatility values.
4. Then experimental recoveries of compounds are used to interpolate relative volatility values.

Example: Using the slide 23 plot as a result of an experiment, an experimental recovery of a compound of 10% (y-axis) would result in assigning the compound a relative volatility value of 20 (x-axis).

Water Interaction Summary

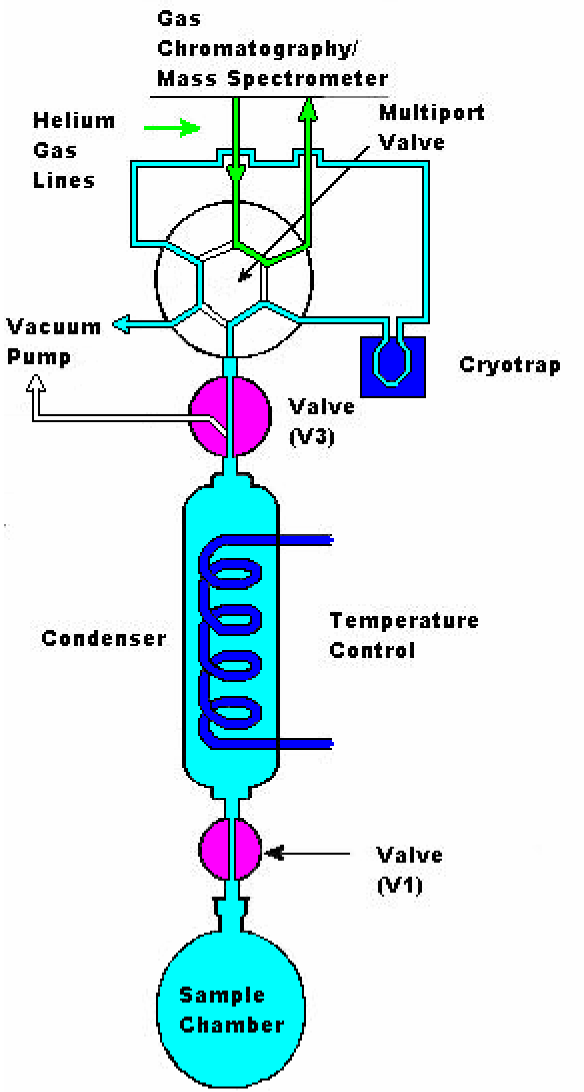
- The potential for a compound to be separated from water into the gas phase during a vacuum distillation is described as relative volatility.
- In method 8261, relative volatility is a physical property closely resembling water-to-air partition coefficients.
- The fraction of a compound separated from water as a gas relates to its recovery.
- Next we'll look at the method 8261 apparatus so we can put all of this information to work.

Vacuum Distillation Components

- There are four fundamental parts to the vacuum distiller: the sample chamber, condenser, cryotrap, and vacuum pump.
- The sample container holds the sample and is of a rigid material to maintain low pressures.
- The condenser is a stainless steel cylinder whose interior surface is temperature controlled and is used to separate water from vapors that pass through it.
- The cryotrap is similar to the condenser though smaller, and its interior surfaces can be made very cold (-196 ºC) to condense vapors that have passed through the condenser. The cryotrap can also be rapidly heated to vaporize material frozen in it. Note: There is no trapping material contained in the cryotrap.
- A mechanical vacuum pump provides the low pressure used in the vacuum distillation. The vacuum in a vacuum distiller is sufficient to lower the pressure in the sample vessel so that water will boil at room temperature.

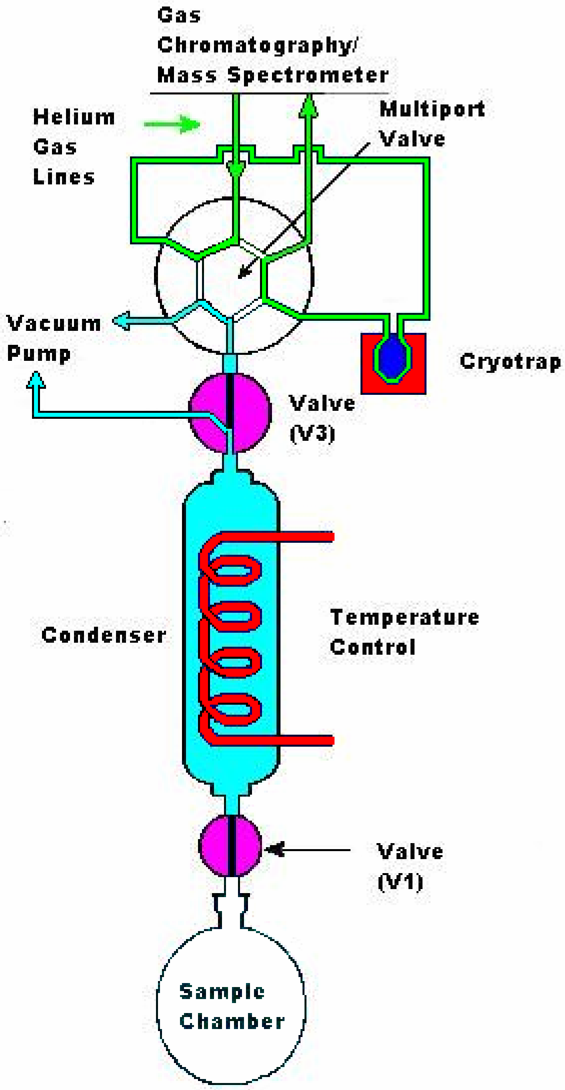
Apparatus During Vacuum Distillation

Multiport valve in vacuum distilling position allows the vacuum distillation of volatile compounds from vessel through condenser and subsequently condensed in the cryotrap. Light blue shows zones under vacuum, dark blue shows cooling zones (condenser = ~ 0 ºC and cryotrap ~ -196 ºC), and green shows helium carrier gas flow.



Apparatus During Transfer of Distillate to GC/MS

Multiport valve in transfer position allows distillate in cryotrap (heated) to vaporize and transfer to GC/MS.
Light blue shows zones under vacuum, red shows heating zones, and green shows helium carrier gas flow.



Vapor Pressure

- Compounds take a long path from the sample to the GC/MS. Losses of analyte that have migrated from the sample chamber occur at the condenser. Because the condenser is held at ~ 0 ºC, losses of compounds due to condensation occur and these losses are related to a compound’s vapor pressure. As noted previously, vapor pressure for a compound is a function of its boiling point, thus we use boiling point as the variable to relate these losses.

Condensation of Compounds in Vacuum Distiller

- A compound must have a boiling point that reflects a vapor pressure giving the compound the ability to migrate from the sample chamber. A compound may not be soluble in water and have an elevated boiling point that makes the compound unlikely to remain a gas. Such compounds condense on the sides of the sample chamber.
- Some compounds may have lower boiling points just sufficient to be partially condensed on the sample chamber walls and even on the lines connecting the components (normally heated to 95 ºC).
- The condenser column is a major site for condensation as it is held at ~ 0 ºC to condense water during the distillation. But because the condenser is also under vacuum, compounds that boil well above 0 ºC are still partially gaseous.
- The cryotrap itself is cooled to a temperature that essentially condenses all analytes that have made their way from the sample chamber.

Condenser

- As noted, the condenser serves to remove a majority of water that is volatilized from the sample during a distillation. This is the major barrier for analytes to pass after they have become gaseous. This function is more thoroughly addressed in the training presentation, “Tuning the Vacuum Distiller, Optimizing Analyte Response and Chromatography” (http://www.epa.gov/nerlesd1/chemistry/vacuum/training/pdf/tuning_vdist.pdf).
- In order to describe how compounds behave during a distillation, losses in the condenser must be well characterized.

Condensation of Compounds in Condenser as a Function of Boiling Point

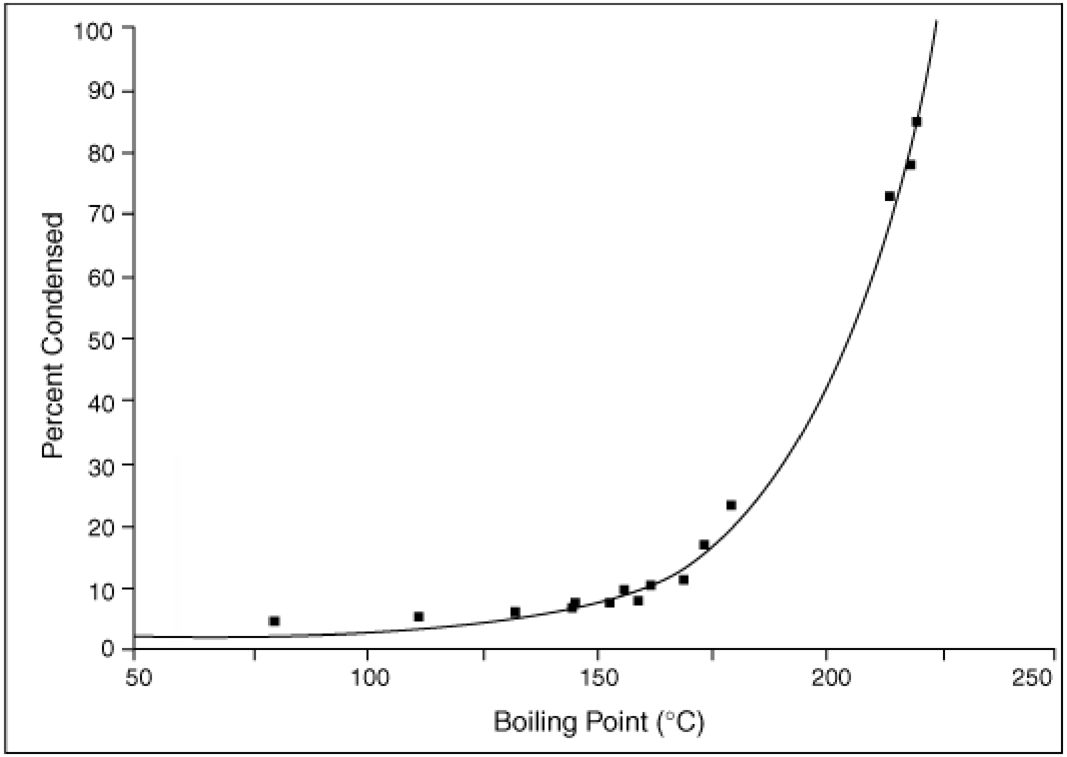


Figure 9. Comparison of boiling points to the trapping of analytes on the condenser column using Table 3 data.

[2]Hiatt, M.H., Farr, C.M, "Volatile Organic Compound Determinations Using Surrogate-Based Correction for Method and Matrix Effects", **Analytical Chemistry,** Vol. 67, No. 2, p. 426-433, January 15, 1995

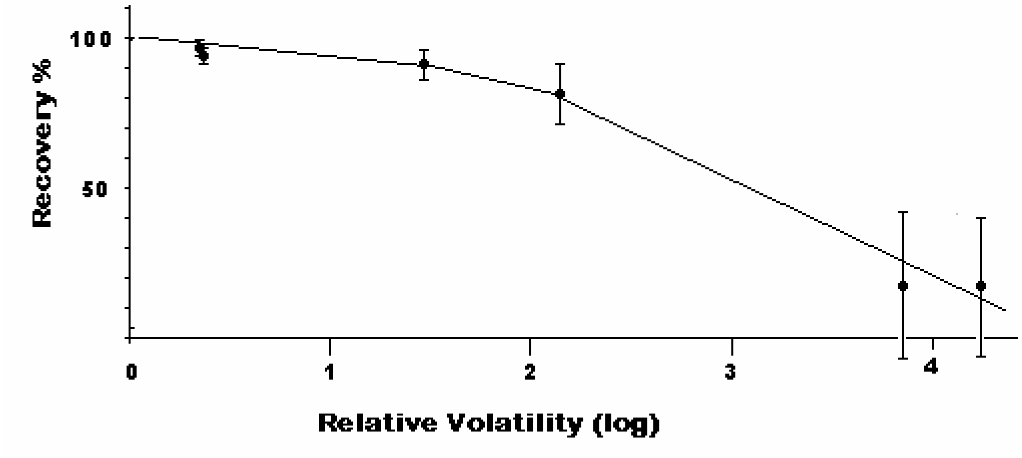
Boiling Point Internal Standards

- The previous plot is characteristic of how condensation, and therefore loss of analyte in the condenser, is a function of a compound’s boiling point.
- Selection of internal standards that have boiling points that cover a range of boiling points allows generating a plot of recovery (total –loss) vs. boiling point.
- Adding these boiling point internal standards to each sample before distillation and then calculating their recovery, yields the data necessary to generate a boiling point vs. recovery plot for every distillation.
- After generating the plot, the prediction of recovery of other compounds is interpolated.

Condenser Partitioning

- By trapping water, the condenser introduces an additional water-to-air partitioning potential.
- The water trapped in the condenser can also dissolve a portion of the water-soluble compounds that were volatilized from a water sample.
- This trapping of compounds relates to the same water-air partitioning that describes their being volatilized from water. The recovery of compounds by volatilization from a water sample and their escaping the condenser water are treated as the same phenomenon using relative volatility.

Trapping in Condenser as a Function of Relative Volatility



[2]Hiatt, M.H., Farr, C.M, “Volatile Organic Compound Determinations Using Surrogate-Based Correction for Method and Matrix Effects”, **Analytical Chemistry,** Vol. 67, No. 2, p. 426-433, January 15, 1995. Note: Altered to reflect recovery vs. method 8261 relative volatility values.

Cryotrap

- The function of the cryotrap is to condense the analytes that have passed through the condenser during vacuum distillation in a volume sufficiently small so that the helium carrier gas can quickly flush the trap and transfer the analytes to the GC.
- The analytes are condensed using liquid nitrogen to obtain cryogenic temperatures (down to -196 ºC).
- After the vacuum distillation is complete, the cryotrap is heated to volatilize and transfer compounds to the GC.

Note: Heating and transfer time of the cryotrap is set to meet gas chromatograph requirements. See the training presentation “Tuning the Vacuum Distiller Optimizing Analyte Response and Chromatography” for more details (http://www.epa.gov/nerlesd1/chemistry/vacuum/training/pdf/tuning_vdist.pdf).

Apparatus Effects

- While the evacuation of a sample induces compounds to volatilize, we can still expect to lose compounds in the apparatus.
- Primarily, losses relate to a compound's boiling point and these losses reflect condensation in the condenser. These losses are measured as functions of boiling point and are measured with boiling point internal standards.
- Any loss of a compound in the condenser that relates to its relative volatility is not distinguished from the fraction of the compound remaining in the sample after vacuum distillation. Relative volatility is a catch-all that describes all forms of loss due to solubility in water.

Diffusion-Warning!

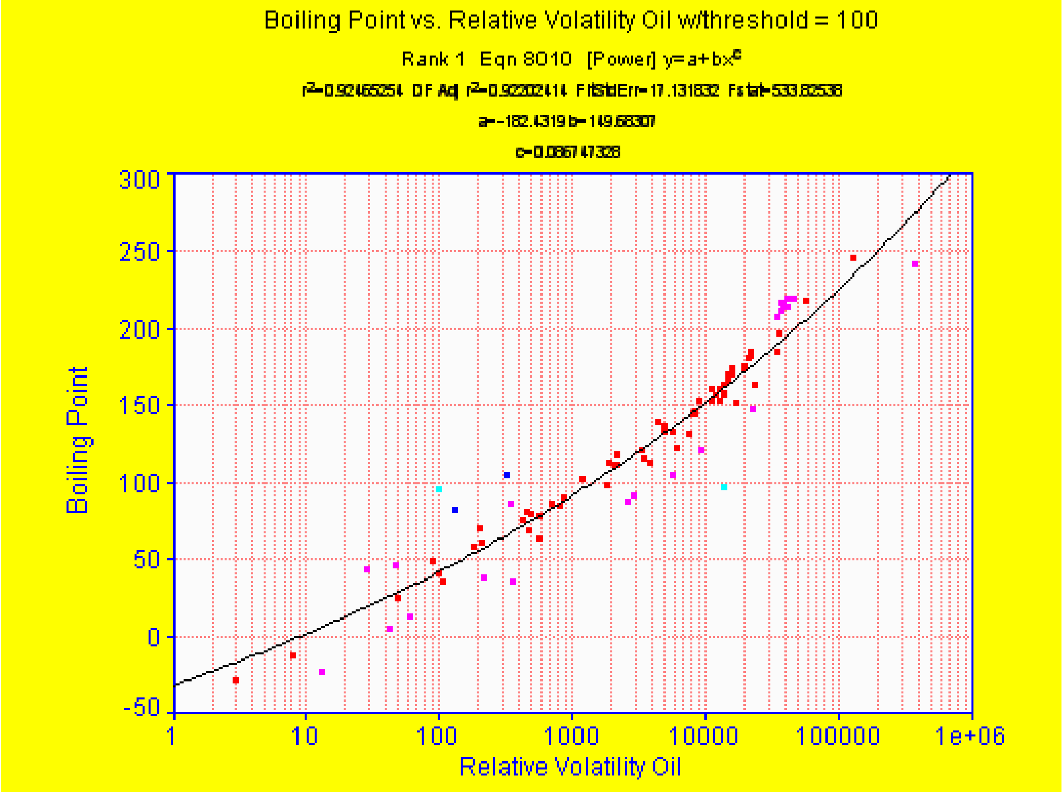
- Diffusion is another variable that must be considered in all volatile organic compound determinations. While we have been discussing water samples, for organic samples this variable is more important. This variable can seriously inhibit the migration of compounds through the sample matrix so that internal standards are not as homogeneous in the matrix as would be analytes.
- Tissue and other organic solids could be expected to limit diffusion of compounds through them. Migration of volatile compounds through organic media can take hours. Therefore the introduction of internal standards should allow ample time for the internal standard to diffuse throughout the sample.
- In organic solids, special precautions such as the "vacuum spike" is recommended to introduce the internal standards. The vacuum spike removes air from a sample container while the sample is cryogenically frozen and the internal standards are much more quickly equilibrated with the matrix. More thorough information is available in the publications section of this website.

[3] Hiatt, "Analyses of Fish Tissue by Vacuum Distillation/Gas Chromatography/Mass Spectrometry", Analytical Chemistry, Vol. 69, No. 6, p. 1127-1134, March 15, 1997 .

Oil Samples

- Water was used as the sample matrix in the previous discussions. However, these findings hold for other matrices with different characteristics.
- In the method 8261 protocol, water is added to oil samples before analysis. We find that the internal standards (boiling point and relative volatility) still accurately describe recovery of compounds.
- The boiling point internal standards also measure the affinity of compounds for oil. In the publication referenced earlier, “Analyses of Fish Tissue by Vacuum Distillation/Gas Chromatography/Mass Spectrometry”, Analytical Chemistry, Vol. *69*, No. 6, p. 1127-1134, March 15, 1997, the relative volatility of compounds in an oil matrix were determined. Comparing this oil-based relative volatility to boiling point we have a good correlation of 0.92 (next slide).
- Because the boiling point internal standards measure recovery from both boiling point condensation and volatilization from oil, method 8261 also applies to oil.

Boiling Point vs. Relative Volatility from Oil



Data from reference 3, Hiatt, “Analyses of Fish Tissue by Vacuum Distillation/Gas Chromatography/Mass Spectrometry”, Analytical Chemistry, Vol. *69*, No. 6, p. 1127-1134, March 15, 1997 .

Soil Samples

- In the method 8261 protocol, water is added to soil samples before analysis and therefore relative-volatility internal standards are needed to describe volatilization from the water phase.
- Soil material is largely inert with the exception of organic matter. The affinity of compounds for the organic matter is also characterized by the boiling point internal standards as seen in the discussion for the oil matrix.
- Again as with the oil matrix, the boiling point internal standards measure recovery from both boiling point condensation and volatilization from organic matter, making method 8261 applicable to soil.[4]

[4] Hiatt, “Vacuum Distillation Coupled with Gas Chromatography/Mass Spectrometry for the Analysis of Environmental Samples” Analytical Chemistry, Vol. *67*, No. 22, p. 4404-4052, 1997 .

Biota Samples

- The relative-volatility internal standards profile the recovery of compounds from the water content of biota (and any added water) while the boiling point internal standards profile the recovery of compounds from the organic phase.
- The dimensions of biota samples may inhibit diffusion of the internal standards and therefore ample time is required to ensure the internal standards are homogeneous in the biota. This is performed by the vacuum spike previously discussed.
- With ample equilibration of internal standards, method 8261 is applicable to biota samples.[3,5,6,7]

[5] Hiatt “Bioconcentration Factors for Volatile Organic Compounds in Vegetation” Analytical Chemistry, Vol. *70*, No. 5, p. 851-856, 1998.

[6] Hiatt “Leaves as an Indicator of Exposure to Airborne Volatile Organic Compounds” Environ. Sci. Technol. 1999, Vol. *33*, p. 4126-4133 .

[7] Hiatt, Pia “Screening Processed Milk for Volatile Organic Compounds Using Vacuum Distillation/Gas Chromatography/Mass Spectrometry” Archives of Environ. Contam. Toxicol., Vol. 46, No. 2, February 2004

Putting it All Together

- We have identified two primary chemical properties, boiling point and relative volatility, that control how much of a compound will make the trip from sample to detector.
- The influence these properties have can be profiled using unique compounds that have been introduced ("spiked") to a sample.
- These properties act independently so that their combined effects are multiplicative products. (e.g., if only ½ of a compound escapes water (relative volatility) and ½ is lost in condenser (boiling point) the total recovery will be ¼).

Method 8261 Internal Standards

- In method 8261, internal standards are added to every sample before a vacuum distillation.
- Their response measured by the GC/MS is compared with a calibrated response to quantify recovery.
- The internal standard recoveries are used to plot recovery vs. relative volatility such as shown in slide 23 and recovery vs. boiling point (slide 33) and these plots predict recoveries of analytes.
- GC/MS responses of analytes are normalized by their predicted recoveries so that they can be reported as concentrations in the sample.
- A stepwise discussion on the process of generating analytical results is addressed in another presentation, "Method 8261 Analytical Reporting" (http://www.epa.gov/nerlesd1/chemistry/vacuum/training/pdf/analytical_report.pdf).

Summary

- Chemical properties affect the recovery of compounds during a vacuum distillation
- The volatilization of compounds from water depends on relative volatility.
- The boiling point of compounds also affects recovery of compounds during a vacuum distillation.
- The addition of internal standards to measure the impact relative volatility and boiling point have on recovery of compounds allows the measurement of method performance.
- The boiling point internal standards also measure the volatility of compounds from an organic phase making method 8261 applicable to most matrices