HUMAN HEALTH

ENVIRONMENTAL HEALTH

AN INTRODUCTION TO HEADSPACE SAMPLING IN GAS CHROMATOGRAPHY

FUNDAMENTALS AND THEORY

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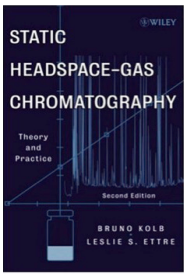
Introduction

This document is intended to provide the newcomer to headspace sampling with a concise summary of the theory and principles of this exciting technique.

Enough information is included here for the user to understand the basic concepts and relationships in HS sampling to apply during method development and interpretation of data. Although emphasis is given to the PerkinElmer TurboMatrix™ HS systems, the document also covers alternative systems so that it should be useful to all potential users of HS systems.



It is not intended to be a comprehensive review of the subject and the reader is directed to an excellent book on this subject by Bruno Kolb and Leslie S. Ettre entitled "Static Headspace-Gas Chromatography"[1]. This book is available for purchase from PerkinElmer under the part number: N101-1210.

Fundamental Theory of Equilibrium Headspace Sampling

Volatile Analytes in a Complex Sample

Headspace sampling is essentially a separation technique in which volatile material may be extracted from a heavier sample matrix and injected into a gas chromatograph for analysis.

To appreciate the principle, let's consider an application that is well suited for headspace sampling: perfume. The composition of perfume may be highly complex containing water, alcohol, essential oils etc. If we inject such a sample directly into a typical GC injector and column, we get the chromatogram shown in *Figure 1*. A lot of time may be wasted in producing this chromatogram by eluting compounds that we have no interest in. Furthermore, many of these compounds may not be suited to gas chromatography and will gradually contaminate the system or even react with the stationary phase in the column so their presence is unwelcome.

If we put a sample of this perfume into a sealed vial and heat it to a moderate temperature (say 60 °C) for a period of time, what happens to the various molecules in the perfume inside the vial?

Consider *Figure 2*. The more volatile compounds will tend to move into the gas phase (or headspace) above the perfume sample. The more volatile the compound, the more concentrated it will be in the headspace. Conversely, the less volatile (and more GC-unfriendly) components that represent the bulk of the sample will tend to remain in the liquid phase. Thus a fairly crude separation has been achieved.

If we can extract some of the headspace vapor and inject it into a gas chromatograph, there will far less of the less-volatile material entering the GC column making the chromatography

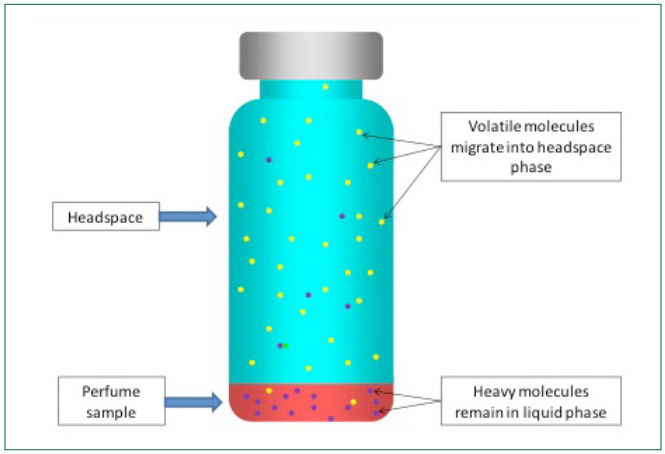


Figure 2. Movement of perfume molecules within a sealed and heated vial.

much cleaner, easier and faster. A headspace sampling system automates this process by extracting a small volume of the headspace vapor from the vial and transferring it to the GC column. *Figure 3* shows a chromatogram produced from a headspace sample taken from the same sample of perfume that produced *Figure 1*.

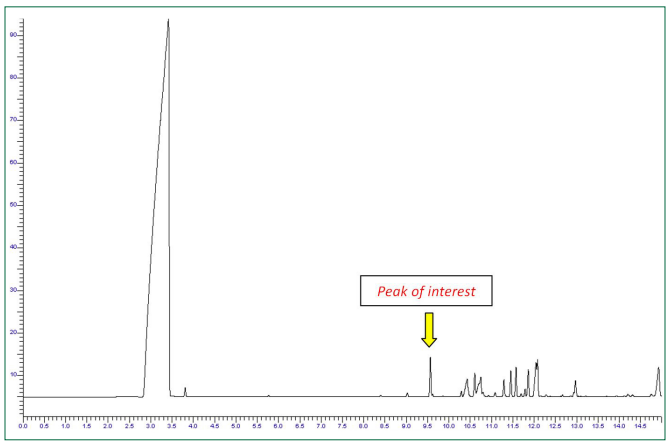


Figure 1. Chromatogram from direct injection of a perfume sample.

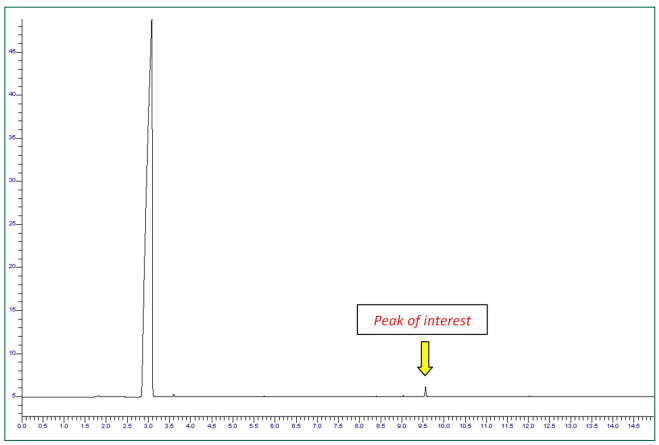


Figure 3. Chromatography of a perfume sample with headspace sampling.

Partition Coefficients

The previous description is simplified. In practice, the migration of compounds into the headspace phase does not just depend on their volatility but more on their affinity for the original sample phase. Furthermore, if the contents inside the sample vial are left long enough, the relative concentrations of a compound between the two phases will reach a steady value (or equilibrium).

For every compound, there is a thermodynamic energy associated with its presence in the headspace phase and in the liquid phase. These thermodynamic properties dictate how the molecules will ultimately distribute themselves between the two phases. The most convenient way of representing this distribution is through the partition coefficient (also known as the distribution ratio), ***K***.

The partition coefficient is proportional to the ratio of the concentration of molecules between the two phases when at equilibrium as shown in *Equation 1*.

$$K = \frac{C_S}{C_G} \quad \text{.....Equation 1}$$

Where:

- ***K*** is the partition coefficient of a given compound between sample (liquid) phase and the gas (headspace) phase
- C_S is the concentration of that compound in the sample (liquid) phase
- C_G is the concentration of that compound in the gas (headspace) phase

Note that compounds with a high value for ***K*** will favor the liquid phase whereas compounds with a low ***K*** will favor the headspace phase. As we want to analyze the headspace phase, we want to ensure that the values of ***K*** for the analytes are much lower than that of unwanted components in the sample matrix. The value of ***K*** will be dependent on both the compound and the sample matrix and it will also be strongly affected by temperature.

Note that this relationship will only apply when the contents in the sample vial are at equilibrium. Thus if this state is attained, then the analytical results should be precise and predictable. This leads to the more formal title for the technique of 'Equilibrium Headspace Sampling' (sometimes also called *'Static Headspace Sampling'*).

It is possible to sample the system when not at equilibrium (and this may be necessary for some samples) but the analytical precision and detection limits may suffer.

Table 1 shows values of ***K*** for a range of compounds in water-air systems at 60 °C [2, 3].

Table 1: Partition coefficients of various compounds between water and air phases at 60 °C.

Compound	K	Compound	K
Dioxane	642	Toluene	1.77
Ethanol	511	o-Xylene	1.31
Isopropyl alcohol	286	Dichloromethane	3.31
n-Butanol	238	1,1,1-Trichloroethane	1.47
Methyl ethyl ketone	68.8	Tetrachloroethylene	1.27
Ethyl acetate	29.3	n-Hexane	0.043
n-Butyl acetate	13.6	Cyclohexane	0.040
Benzene	2.27		

To further explain the meaning of ***K***, let's look at two extremes in *Table 1*: ethanol and cyclohexane. A value for ***K*** of 511 for ethanol means that there is 511 times the volumetric concentration of ethanol in the liquid than in the headspace. This is expected because of the significant hydrogen bonding between the alcohol and water hydroxyl groups. On the other hand, cyclohexane, which does not exhibit any significant hydrogen bonding, has a ***K*** of 0.04 which means the opposite is true; there is approx 25 (inverse of 0.04) times higher concentration in the headspace. In summary, if ***K*** is less than 1 then the analyte favors the headspace while if ***K*** greater than 1, the analyte favors the liquid phase

In practice, this means that it should be easy to use headspace sampling to extract light hydrocarbons from water and more difficult to extract alcohols from water – this provides the theoretical justification to an observation that is rather intuitive anyway.

Phase Ratio

Other factors that can affect the concentration of an analyte in the headspace phase are the respective volumes of the sample and the headspace in the sealed vial.

The concentration of analyte in the sample and the headspace can be expressed respectively as *Equations 2 and 3.*

$$C_S = \frac{M_S}{V_S} \quad \textbf{Equation 2} \qquad C_G = \frac{M_G}{V_G} \quad \textbf{Equation 3}$$

Where:

- C_S is the concentration of compound in the sample (liquid) phase
- C_G is the concentration of compound in the gas (headspace) phase
- M_S is the mass of compound in the sample (liquid) phase
- M_G is the mass of compound in the gas (headspace) phase
- V_S is the volume of the sample (liquid) phase
- V_G is the volume of the gas (headspace) phase

When the vial contents are at equilibrium, *Equations 2 and 3* may be substituted into *Equation 1* to give *Equation 4.*

$$K = \frac{M_S V_G}{M_G V_S} \quad \textbf{Equation 4}$$

The ratio of the two phase volumes may be expressed as the phase ratio as shown in *Equation 5.*

$$\beta = \frac{V_G}{V_S}$$

Where:

- β is the phase ratio

Substituting *Equation 5* into *Equation 4* gives *Equation 6.* This equation shows us how the mass of a compound will be distributed through the two phases if we know the phase ratio and the partition coefficient.

$$K = \frac{M_S}{M_G} \cdot \beta \quad \textbf{Equation 6}$$

Equation 6 shows how the masses will be distributed but for a chromatographic analysis we need to find a relationship that will enable us to relate the GC detector response to the concentration of a compound in the original sample.

The mass of compound in the original sample will be the sum of the masses in the two phases at equilibrium as shown in *Equation 7.*

$$M_0 = M_S + M_G \quad \textbf{Equation 7}$$

Where:

- M_0 is the total mass of compound in the original sample before analysis

The three compound masses in *Equation 7* may be related to the phase concentrations and volumes by *Equations 8* to *10.*

$$M_0 = C_0 \cdot V_S \quad \textbf{Equation 8}$$

$$M_S = C_S \cdot V_S \quad \textbf{Equation 9}$$

$$M_G = C_G \cdot V_G \quad \textbf{Equation 10}$$

Where:

- C_0 is the concentration of compound in the original sample before analysis

Substituting *Equations 8 to 10* into *Equation 7* gives *Equation 11.*

$$C_0 \cdot V_S = C_S \cdot V_S + C_G \cdot V_G \quad \textbf{Equation 11}$$

The compound concentrations in each phase may be related to the partition coefficient by *Equation 12,* which is a re-arrangment of *Equation 1.*

$$C_S = K \cdot C_G \quad \textbf{Equation 12}$$

Substituting *Equation 12* into *Equation 11* gives *Equation 13*

$$C_0 \cdot V_S = K \cdot C_G \cdot V_S + C_G \cdot V_G \quad \textbf{Equation 13}$$

Rearranging *Equation 13* gives *Equation 14.*

$$C_0 = C_G \cdot \left[K \frac{V_S}{V_S} + \frac{V_G}{V_S} \right] \quad \textbf{Equation 14}$$

Equation 14 may be further manipulated to give *Equation 15*

$$C_G = \frac{C_o}{(K+\beta)} \quad \text{.....} \textbf{Equation 15}$$

Equation 15 is one of the key relationships in equilibrium headspace sampling. It tells us the following:

- If we increase the sample volume, V_s, we will reduce the headspace volume, V_G, in the same vial and so **β** will be reduced as a result. Decreasing β will increase the concentration of all compounds in the headspace phase.
- If we decrease ***K,*** for instance by raising the vial temperature, then this will have the effect of pushing more compound into the headspace. Of course more of the sample matrix will also pass into the headspace and there is a risk of increasing the pressure inside the vial that affects the sampling process or even cause leakage or breakage in extreme cases.
- If we keep ***K*** and **β** consistent between samples and calibration mixtures, then the compound concentration in the headspace vapor (and thus the chromatographic peak area) will be directly proportional to its concentration in the sample prior to analysis.
- It helps us predict the impact of changing ***K*** and/or **β** on the observed chromatographic peak size.

Vapor Pressures and Dalton's Law

So far, in this discussion we have assumed that the value of ***K*** is constant for a given compound. This should be the case if the temperature and the sample matrix are consistent. While this is true for dilute solutions, inter-molecular interactions may cause deviations at higher concentrations. To understand this further we need to consider the relationship between ***K*** and vapor pressure.

If we were to examine the composition of the headspace vapor from a complex liquid sample that has been sealed and thermally equilibrated inside a suitable vial, we would find a variety of compounds present. Each compound vapor will contribute to the total pressure observed inside the vial. *Dalton's Law of Partial Pressures* states that the total pressure exerted by a gaseous mixture is equal to the sum of the partial pressures of each individual component in a gas mixture. At equilibrium, the partial pressure of a compound will be equivalent to the vapor pressure of that compound. This relationship can be expressed as *Equation 16.*

$$p_{total} = \Sigma p_i \quad \text{.....} \textbf{Equation 16}$$

Where:

p_{total} is the total pressure of the headspace vapor
p_i is the partial pressure of component i

The partial pressure of each component in the headspace is proportional to the fraction of its molecules in the total molecules present as shown in ***Equation 17.***

$$p_i = p_{total} \cdot x_{G(i)} \quad \text{.....} \textbf{Equation 17}$$

Where:

$x_{G(i)}$ is the mole fraction of compound i in the headspace vapor

Because the concentration of a compound in the headspace vapor is directly proportional to the number of molecules of it present, we can say that its concentration is proportional to its partial pressure.

Raoult's Law

Raoult's Law states that the vapor pressure of a compound above a solution is directly proportional to its mole fraction in that solution as shown in *Equation 18*.

$$p_i = p_i^0 \cdot x_{s(i)} \quad \text{Equation 18}$$

Where:

p_i^0 is the vapor pressure of the pure compound i in the headspace vapor

$x_{s(i)}$ is the mole fraction of compound i in the liquid phase

In essence, *Equation 18* tells us that the concentration of a compound in the vapor phase is proportional to its concentration in the liquid phase.

This relationship may be depicted graphically as shown in *Figure 4*. The compound concentration and the resultant GC peak area will be proportional to its vapor pressure.

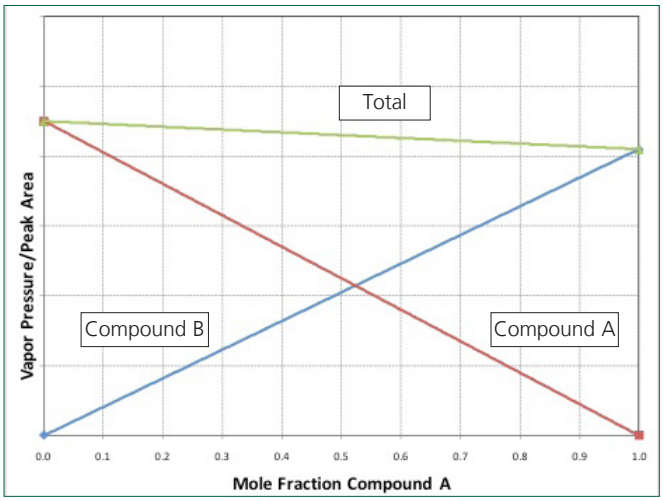


Figure 4. Relationship between partial pressures and mole fractions in an ideal binary mixture.

Activity Coefficients

Equation 18, however, assumes that the components in the mixture behave in an ideal manner. In practice this rarely occurs because molecules may interact with each other and have a consequential effect on the vapor pressure. To accommodate these deviations from the ideal, Raoult's Law is modified to include activity coefficients as shown in *Equation 19*.

$$p_i = p_i^0 \cdot \gamma_i \cdot x_{s(i)} \quad \text{Equation 19}$$

Where:

γ_i is the activity coefficient of the compound ***i*** in the sample mixture

In a binary mixture, there are types of 3 molecular interactions:

- Between molecule A and molecule A
- Between molecule B and molecule B
- Between molecule A and molecule B

If the nature of these interactions is similar in all three instances, then the value of γ_i would be close to 1 and *Equation 18* and *Figure 4* would apply. An example would a mixture of compounds with the same molecular structure but containing different isotopes.

If the molecular attractions are stronger between different molecules than within the pure compounds, then the value of Compound A would become and give rise to a partial pressure relationship as illustrated in *Figure 5* in which hydrogen bonding is higher between dissimilar molecules in a mixture of chloroform and acetone [4].

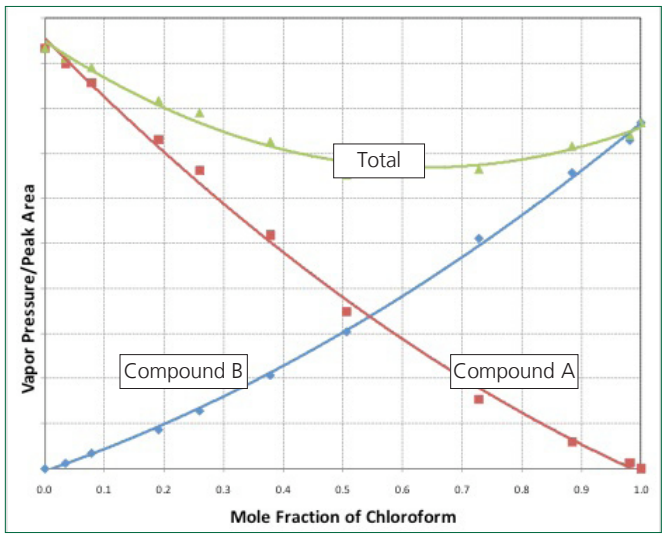


Figure 5. Relationship between partial vapor pressures and mole fractions in a mixture of chloroform and acetone with negative activity coefficients.

So what does all this mean with respect to the value of the partition coefficient, ***K***?

By combining *Equation 17* with *Equation 19,* we can derive *Equation 20*.

$$K = \frac{p_{total}}{p_i^0 \cdot \gamma_i} \quad \text{Equation 20}$$

If the molecular attractions are weak between different molecules than within the pure compounds, then the value of γ_i would become positive and give rise to a partial pressure relationship as illustrated in *Figure 6* for a mixture of n-hexane and ethanol [4].

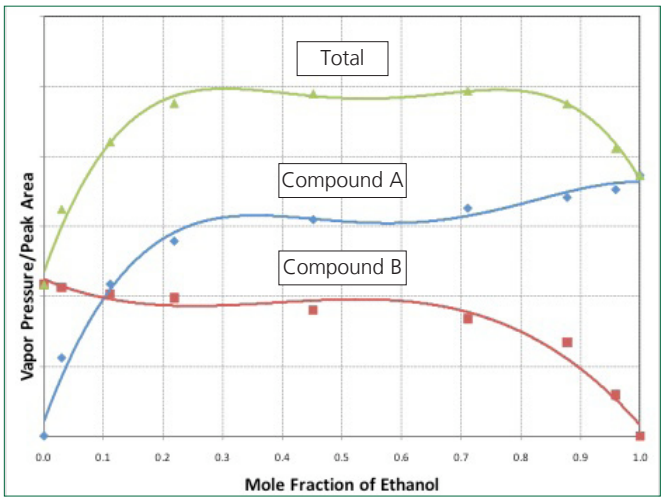


Figure 6. Relationship between partial pressures and mole fractions in a mixture of n-hexane and ethanol with positive activity coefficients.

Henry's Law

Note that the value of $\boldsymbol{\gamma_i}$ may change with concentration. In a dilute solution with concentrations less than approximately 0.1%, the molecular interactions for a compound will be almost exclusively with other molecules in the sample matrix and not with those of itself. This has the effect of making $\boldsymbol{\gamma_i}$ and hence *K* effectively constant over a range of applied conditions. Under these conditions, *Henry's Law* will apply. This states that, at a constant temperature, the amount of a gas dissolved in a liquid is directly proportional to the partial pressure of that gas at equilibrium with that liquid. This can be expressed mathematically by *Equation 21*.

$$\boldsymbol{p_i = H_i \cdot x_{s(i)}} \quad \textbf{Equation 21}$$

Where:

$\boldsymbol{p_i}$ is the is the Henry's Law constant for compound ***i*** in the sample matrix

Note that although *Equation 21* looks very similar to *Equation 18* and *Equation 19*, it will only be equivalent if the activity coefficient is unity. In all other instances, *Equation 21* will only apply at the extremes of the charts shown in *Figure 5* and *Figure 6*.

Because analysis involving headspace sampling and gas chromatography is normally looking at analyte concentrations well below 0.1%, in the vast majority of applications, *Henry's Law* will apply and we can assume that ***K*** will be constant across the range of concentrations to be monitored and thus that the concentration in the headspace will be proportional to the original concentration in the sample.

At higher concentrations, some non-linearity in the response curve is to be expected because the activity coefficients will vary and so the analysis will require a multi-level calibration with curve fitting for accurate quantification.

Putting It All Together

So what do all these equations mean to the chromatographer?

In the following discussion, we will assume that we are dealing with the analysis of components at low concentrations and so ***K*** will not change with different concentrations.

Effect of Sample Volume

Equation 15 shows us that the concentration of a compound in the headspace vapor phase is proportional to its original concentration in the sample and the reciprocal of the partition coefficient ***K*** added to the phase ratio **β**. If ***K*** is low (the compound prefers the headspace phase), then the value of **β** (hence sample volume), significantly affects the concentration in the headspace phase. Conversely if ***K*** is high (the compound favors the sample phase) then adjusting **β** will have a minor effect on the concentration in the headspace phase.

The effect of adjusting the sample volume in a typical vial on concentration in the headspace phase for three compounds with high, medium and low partition coefficients is illustrated graphically in *Figure 7, Figure 8* and *Figure 9* respectively (note the different scaling of the y-axes for each of these).

In the case of a compound with a high partition coefficient such as ethanol in water as shown in *Figure 7* the effect of changing the sample volume makes little difference to the concentration in the headspace vapor. In instances where sample is in short supply (e.g. forensic samples), lower volumes may be used with no significant loss in performance. Note that although the concentration and GC response will be largely independent of the sample volume, there will still be proportionality between the sample concentration and the concentration in the headspace vapor.

In situations with a medium value for ***K*** as seen for toluene in water as shown in *Figure 8,* there is an approximately proportional relationship between sample volume and headspace concentration.

With a very low value for ***K*** as shown for n-hexane in *Figure 9,* a small change in the sample volume makes a big difference in headspace concentration. In these instances, analytical detection limits are greatly enhanced by an increase in sample volume. Note that is it even possible to create a headspace with a higher concentration of the compound than originally in the sample.

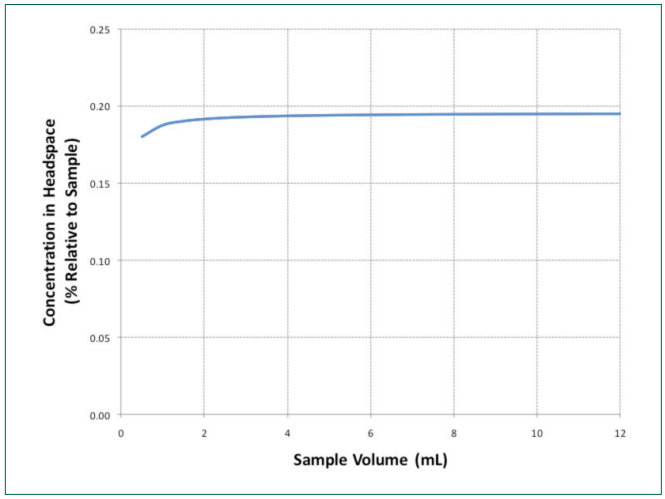


Figure 7. Headspace concentration versus sample volume for ethanol in water at 60 °C (K=511) in a 22 mL vial.

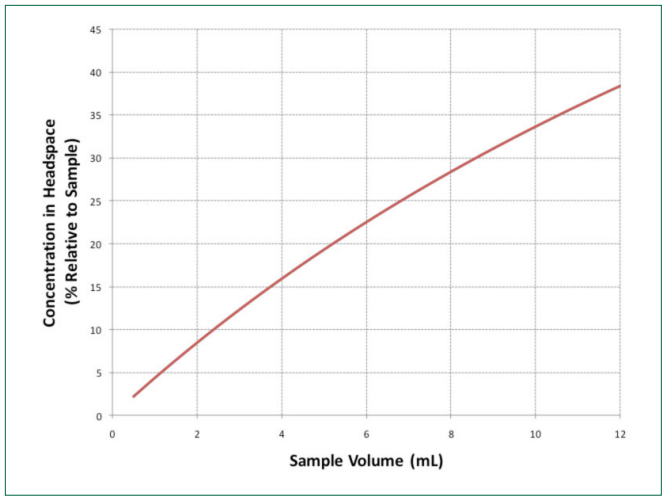


Figure 8. Headspace concentration versus sample volume for toluene in water at 60 °C (K=1.77) in a 22 mL vial.

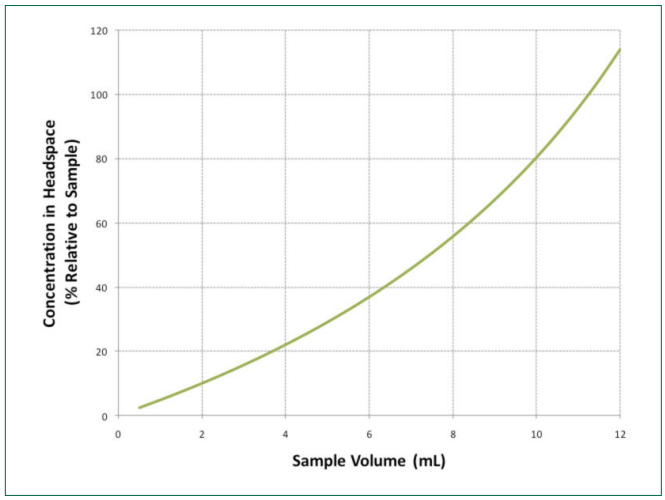


Figure 9. Headspace concentration versus sample volume for n-hexane in water at 60 °C (K=0.043) in a 22 mL vial.

Effect of Temperature

The partition coefficient of a compound in the sample is related to the inverse its vapor pressure when pure as shown in *Equation 20*. Vapor pressure increases with temperature and so the value of ***K*** will decrease and more of the compound will pass into the headspace phase. This observation is very intuitive – hot liquids will quickly release dissolved volatile compounds.

Table 2 is an extension to *Table 1* showing partition coefficients over a range of temperatures.

Table 2. Partition coefficients of various compounds between water and air phases over a range of temperatures [2, 3].

Compound	40 °C	60 °C	70 °C	80 °C
Dioxane	1618	642	412	288
Ethanol	1355	511	328	216
Isopropyl alcohol	825	286	179	117
n-Butanol	647	238	149	99
Methyl ethyl ketone	139.5	68.8	47.7	35.0
Ethyl acetate	62.4	29.3	21.8	17.5
n-Butyl acetate	31.4	13.6	9.82	7.58
Benzene	2.90	2.27	1.71	1.66
Toluene	2.82	1.77	1.49	1.27
o-Xylene	2.44	1.31	1.01	0.99
Dichloromethane	5.65	3.31	2.60	2.07
1,1,1-Trichloroethane	1.65	1.47	1.26	1.18
Tetrachloroethylene	1.48	1.27	0.78	0.87
n-Hexane	0.14	0.043	0.012	
Cyclohexane	0.077	0.040	0.030	0.023

Data from *Table 2* for ethanol, toluene and n-hexane are plotted graphically in *Figure 10, Figure 11* and *Figure 12* respectively (note the different scaling of the y-axis in each of these).

From *Figure 10* we see that the headspace concentration is highly affected by a change in temperature for a compound like ethanol with high values of ***K*** when in water. This chart underlines the need for careful temperature control of the vial during the equilibration step. For instance if the temperature of the vial drifted by only 1 °C from a set temperature of 60 °C, the change in concentration of ethanol in the headspace would change by almost 5%. To achieve a quantitative precision of 0.5% (which is typical for a good headspace sampling system) the temperature of the vial must be controlled to within 0.1 °C.

For medium values of ***K,*** the relationship is approximately proportional as shown in *Figure 11*.

When ***K*** is low, there is only minor change in the headspace concentration as the temperature is raised as shown in *Figure 12*.

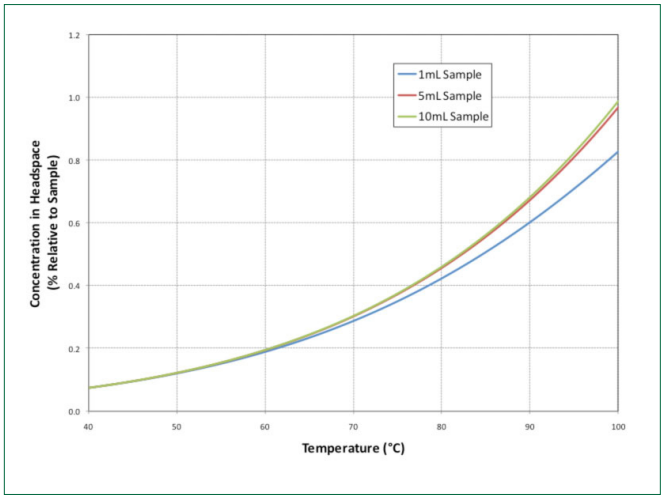


Figure 10. Headspace concentration versus temperature for ethanol in water.

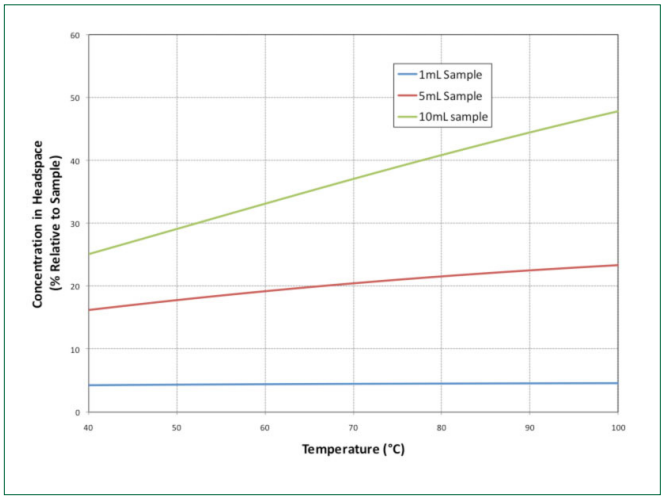


Figure 11. Headspace concentration versus temperature for toluene in water.

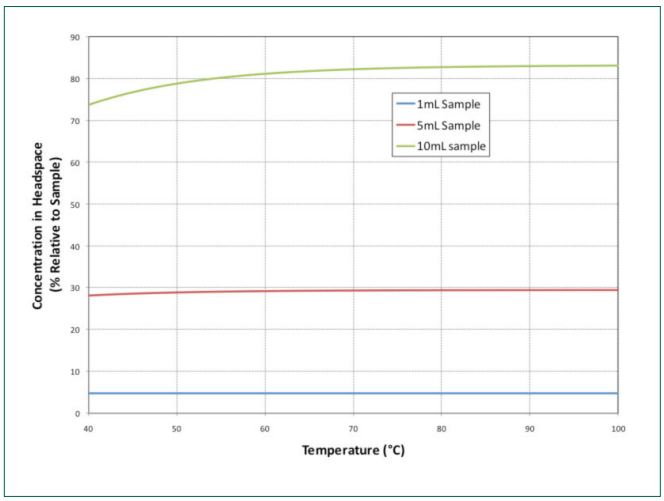


Figure 12. Headspace concentration versus temperature for n-hexane in water.

One important aspect that must be considered when changing temperature is the effect on the vapor pressure of the sample matrix. In the case of water, which is present in most sample matrices, the vapor pressure increases with temperature as shown in *Figure 13*.

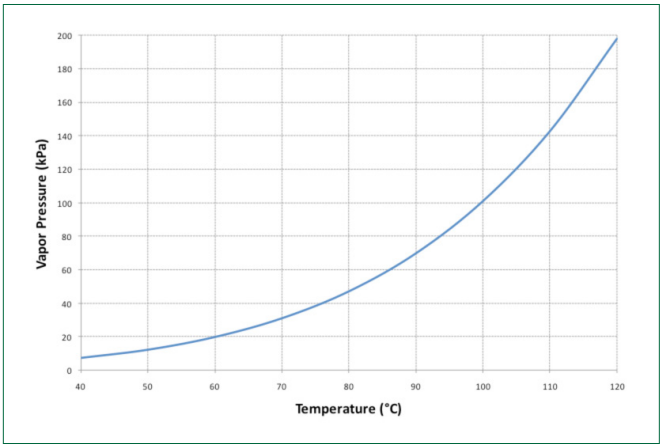


Figure 13. Vapor pressure of water versus temperature [5].

As stated at the beginning of this document, headspace sampling is essentially a separation technique in which we try to extract and inject the volatile components and leave the bulk of the less-volatile sample matrix in the sample vial.

For nearly all compounds, the concentration ratio of a compound in water to that in the headspace vapor will increase proportionally as the sample temperature is increased. This relative increase is most pronounced with compounds with a low value of ***K.***

Thus although increasing temperature can be a very effective way of increasing analyte concentration in the headspace vapor, especially for compounds with a high value of ***K,*** there will still be a significant increase in the amount of water vapor in the headspace vapor. If a column or detector is particularly susceptible to the presence of water then caution must be exercised before increasing the temperature significantly.

Also note that a heated liquid inside a sealed vial can build up a significant vapor pressure which could easily exceed the pressure rating of a sample vial, so check the vial specifications carefully before proceeding.

Over-pressurizing can also lead to premature injection of the headspace vapor giving it a double peak effect in the chromatography.

Effect of Pressure

Equation 17 shows us that the concentration of a compound in the headspace phase is proportional to its partial pressure in the headspace phase.

To establish the partial pressure, we must first determine what the total pressure of the headspace vapor will be.

The pressure inside a sealed sample vial will increase through one of two reasons:

- Its temperature is increased. This will increase the vapor pressure of any liquids in the sample and will increase the pressure of the air inside the vial when it was sealed.
- Carrier gas is added for sampling purposes. On some systems, including those that use the PerkinElmer pressure-balanced sampling technique, carrier gas is used to pressurize the sample vial to an elevated pressure immediately prior to sampling. Thus the headspace vapor contains vapors from the sample, air that was present inside the vial when it was sealed and an amount of carrier gas necessary to attain a pressure inside the vial required for sampling purposes.

For the purposes of this discussion, we will focus on the second reason as this is what will be occurring on the PerkinElmer headspace sampling systems.

When carrier gas is injected into the sample vial to raise the pressure, the partial pressure of the compound vapor does not change; neither does the volume of the headspace vapor. Thus although the compound concentration decreases when expressed in terms of a mole fraction, the concentration when expressed as weight/volume remains the same. We typically express concentrations in headspace sampling as ppm w/v or ppb w/v and so, if we think in these terms, the act of injecting carrier gas into the vial has no effect on the concentration of analyte vapors in the headspace phase.

Once the headspace vapor is withdrawn from the vial, it will decrease in pressure as it passes down the transfer line and the column. This expansion will effectively cause a dilution when the concentration is expressed in terms of weight/volume and, depending on the injection technique, may affect the amount of a compound injected into the column and detector. This effect will be more significant with higher vial pressures.

Effect of Modifying the Sample Matrix

The activity coefficients discussed in *Equation 19* may be adjusted in many cases by the addition of salts or solvents to the sample matrix. These modifiers are chosen to increase the activity coefficients and so decrease the partition coefficients and cause more of the compound to pass into the headspace phase.

Table 3 shows the potential benefit of adding inorganic salts to water samples that are to be analyzed for ethanol content. The presence of these salts changes the nature of the molecular interactions; there is now far more ionic activity. This causes an increase in the activity coefficient of ethanol with a resultant increase in the concentration of it in the headspace phase.

Table 3. Potential increase in the concentration of ethanol in the headspace phase after adding salt modifiers to water samples.

Salt	Increase in Concentration
Ammonium sulfate	x5
Sodium chloride	x3
Potassium carbonate	x8
Ammonium chloride	x2
Sodium citrate	x5

Table 4 shows the results of an experiment to investigate the effect of adding water to solutions of various analytes in dimethylformamide (DMF). This is an important application for headspace sampling as it enables residual solvents to be monitored in pharmaceutical preparations.

Table 4. Effect on relative headspace concentration of adding water to 120 ppm solutions of various analytes in DMF.

% Water in DMF	Acrylonitrile	n-Butanol	Butyl Acrylate	Styrene
0	12	2	3	4
10	18	3	7	9
20	25	5	15	21
30	45	9	51	81
40	58	14	83	144
50	71	18	122	227
60	87	23	179	344
70	105	30	243	458
80	118	37	280	556
90	119	45	307	504
100	139	51	334	600

As can be seen in *Table 4*, the addition of water to the DMF solvent drastically increases the concentration of analytes in the headspace phase – over two orders of magnitude in some cases. The presence of water has a major impact on the inter-molecular activity within the same causing a very large increase in the activity coefficients causing these apparent increases in headspace concentration.

Effect of the Equilibration Time

One other factor that should be considered at this point is the equilibration time. The preceding discussion talks about the partition coefficient and its role in dictating the final compound concentration in the headspace phase for a given set of applied conditions.

Partitioning is a process that takes a finite time to complete. Molecules need to move around within the sample phase and headspace phase and between the two. The two most time consuming factors are the molecular diffusion within the liquid sample phase and the mass transfer across the phase boundary.

It is difficult to model this kinetic behavior mathematically and so in most instances, experiments need to be performed to establish the necessary equilibration time. This normally involves the analysis of a series of identical mixtures with known amounts of added analyte(s). The analytical conditions are the same for each analysis except that the equilibration time is incremented between successive runs. At the end of this sequence, a plot of peak response versus equilibration time for each analyte is created and the point beyond which the response no longer increases is established. This is illustrated in *Figure 14*. In this example, as the thermostatting time is increased, we see that the GC response has maximized at about 6 minutes. We would normally set the equilibration time in the method a little longer than this, for instance say 8 minutes, to allow for possible variations in the heat transfer into the vial.

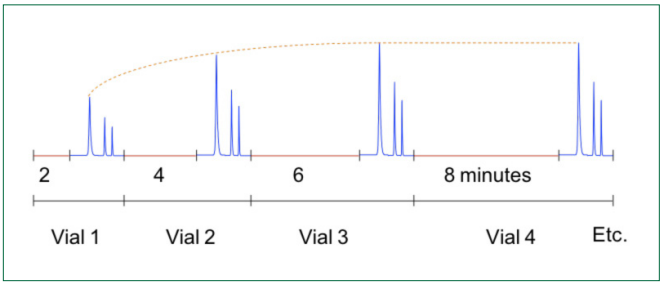


Figure 14. Effect of increasing the thermostatting time.

The type of experiment illustrated by *Figure 14* would be required for method development for each new type of sample. A series of methods would be run with increasing equilibration times. Some systems, including the TurboMatrix, will automatically perform such a sequence from a single method and progressively increment the equilibration time for a given number of samples.

The equilibration time may be long – over one hour in some instances. In most applications, the equilibration time is actually longer than the GC analysis time. To minimize this effect on sample throughput, many instruments will allow multiple vials to be simultaneously equilibrated. By staggering the loading of the vials into the oven, the equilibration time may be applied consistently and have the next vial ready for sampling once the

current analysis is complete. This overlapped thermostatting mode of operation is illustrated in *Figure 15*.

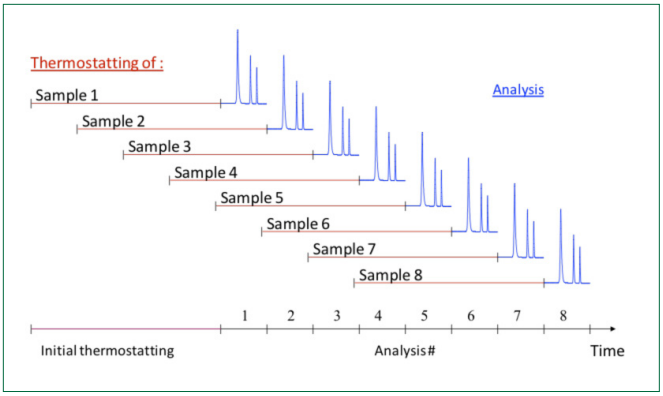


Figure 15. Illustration of the overlapped thermostatting process. Samples to be analyzed may be thermostatted during the chromatography of previous samples.

To enable overlapped thermostatting requires a vial oven with sufficient capacity to handle the number of vials and the thermal mass and heater design to ensure precise and uniform temperature control. *Figure 16* shows the vial oven used on the TurboMatrix systems.

Figure 16. Photograph of a 15-position vial oven taken from a TurboMatrix HS system. This has the capability to overlap the thermostatting of up to 12 vials.

The equilibration time will be reduced if the temperature is increased or if the sample volume is decreased but the most effective means of decreasing the equilibration time is through active shaking of the sample vial contents. This keeps the liquid mixed and so reduces the dependency on diffusion for the molecules to reach the phase boundary. It also dramatically increases the effective area of the phase boundary which helps promote mass transfer across the phase.

Specialized HS Injection Techniques

The Total Vaporization Technique

For some applications, equilibration headspace sampling is unable to extract a sufficient amount of an analyte for analysis. An example would be when the partition coefficient, ***K,*** is so high in the sample that very little of the analyte passes into the headspace phase.

Raising the temperature will reduce the value of ***K,*** as we have seen, but it will also increase the pressure inside the sample vial because the air inside it when sealed and also because of the increased vapor pressure of the sample matrix. Once the temperature passes that of the normal boiling point of the sample, care must be taken not to over-pressurize the vial and cause busting or rupture of the seal. Check the pressure specifications for the vials being used. For PerkinElmer vials, a safety seal mechanism vents the vial at 500 kPa (~75 psig).

There may be a separate need to check or calibrate the system by making simple injections of standard solutions into the vial because of difficulties in reproducing a particular sample matrix – especially for solid samples.

One solution to both these needs is to use the Total Vaporization Technique (TVT). A small amount of sample is added to the sample vial which is then heated to a sufficient temperature to vaporize the whole sample inside the vial. This volume of sample must be low enough not to cause the pressure inside the vial to burst the vial when vaporized. In most applications this means that the volume must be limited to about 13 to 15 µL. In TVT, the vial is effectively used in the same way as a disposable injector liner. *Figure 17* illustrates the principle.

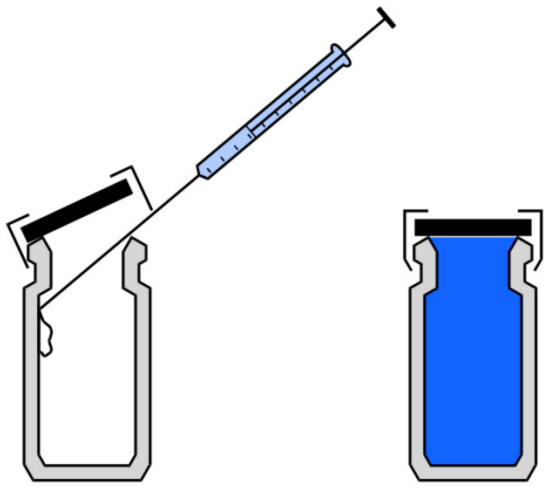


Figure 17. Adding a small amount of liquid sample to an empty vial for the Total Vaporization Technique.

The total amount of analyte added to the vial is the product of its volume and concentration as shown in *Equation 22*.

$$M_S = C_O \cdot V_S \quad \text{Equation 22}$$

Where:

M_S is the mass of compound in the sample (liquid) phase
V_S is the volume of the sample (liquid) phase
C_0 is the concentration of compound in the original sample before analysis

Because the whole sample is vaporized, its volume will occupy the capacity of the sample vial and so the concentration in the gas phase will be as shown in *Equation 23*.

$$C_G = \frac{C_O \cdot V_S}{V_V} \quad \text{Equation 23}$$

Where:

V_V is the capacity of the sample vial

Care must be taken that the analyte has sufficient vapor pressure at the applied temperature to ensure that all of it becomes vaporized. If this is not the case, then part of the analyte will remain in a condensed form and create vapor concentrations that are lower than expected.

Note that although vaporization of a small volume of sample may be expected to be almost instantaneous, in practice it does take time for the vapor concentrations to reach their expected levels. Some time for evaporation and equilibration is still required and needs to be determined experimentally.

The Full Evaporation Technique

The Full Evaporation Technique (FET) is very similar to the TVT technique except that it is used in instances where complete vaporization of the sample cannot be achieved.

In this technique, a low volume of sample is injected, evaporated and thermally equilibrated in a sealed vial. Because the sample is so relatively small, the value of the phase ratio will be large and so, from a practical perspective, all the analyte can be assumed to have passed into the vapor phase. *Equation 4* may be re-arranged to give *Equation 24*.

$$\frac{M_G}{M_S} = \frac{\beta}{K} \quad \text{Equation 24}$$

Where:

M_S is the mass of compound in the sample (liquid) phase
M_G is the mass of compound in the gas (headspace) phase
K is the partition coefficient
β is the ratio of the volumes of the two phases (V_g/V_s)

Study of *Equation 24* indicates that if β is very large, then the ratio between M_G and M_S is also going to be very large so as to make the value of M_S effectively insignificant.

For instance, a sample injection of 10 µL into a 22 mL vial gives a value for β of 2199. If the vial is heated and 50% of the sample evaporates, then the value of β increases to 4399. To get at least 90% of the analyte into the vapor phase, the value of K must then be less than 439.9. Most analytes will achieve this requirement easily - especially at raised temperatures.

Because the sample matrix is no longer significant in terms of how the analyte is distributed within the vial, the analyte concentration is effectively described by *Equation 25*.

$$C_G = \frac{C_O \cdot V_S}{(V_V - V^*_S)} \quad \text{Equation 25}$$

Where:

V^*_S is the remaining volume of the sample that has not been evaporated. In many instances this term can be neglected.

The notes given in the TVT section concerning the maximum sample volume and the need for an equilibration time equally apply to FET.

Multiple Headspace Extraction

So far, we have been discussing the analysis of simple liquid samples. Liquids are generally homogenous and easy to dispense in known volumes. The contents of compounds contained within them are generally expressed in terms of concentration. For the purposes of headspace analysis, it is relatively easy to ensure that there is consistency between the samples so that compounds will partition in the same way.

Headspace sampling can also be applied to other sample types such as soils, polymers, fabrics, gels, emulsions, powders, etc. In many of these instances, the analysis is conducted to find out the total amount of a compound in a given sample. This may be because the sample is not homogenous or because there is large a variation in the matrix between samples that effects the partitioning process.

One technique designed to establish the total amount of a compound in a given sample is a discontinuous extraction process termed Multiple Headspace Extraction (MHE).

In MHE, the sample is normally weighed into a sample vial which is then sealed and thermally equilibrated in the same way as for a regular equilibrium headspace analysis. The equilibration time must be sufficient to enable the components released from the sample to achieve a stable concentration within the vapor phase. The compounds in the sample need to migrate through the sample matrix before they can reach the headspace phase – this will take time. The equilibration time will be significantly reduced if the sample is reduced to granules prior to addition to the vial. Although the sample may be dry, component vapors may still interact with it through sorption effects so a sufficient equilibration time is critical.

MHE works by re-analyzing the same sample multiple times. In between each analysis, the headspace is vented so that the vapor equilibration has to be re-established. For each analysis there is less of the analyte in vial and so the chromatographic peaks get progressively smaller. If we keep re-analyzing the same sample, there will come a point when all the analyte has been effectively withdrawn from the vial. If we were to sum the results from all these repeat analyzes, we would have a measure of the total amount of analyte in the vial and hence in the sample.

To get complete extraction of an analyte from a vial may take very many repeat analyses (in theory, this will be infinite) and so make the technique impractical. However, study of the way that the amount of analyte decreases between successful analyses indicates that there is a mathematical trend in which data from just a few runs may be used to predict the results for further analyses. In this way, a few analyses may be performed and the results from these can be used to estimate the results from all the analyses needed to extract the total analyte from the vial and so provide an estimate of the total analyte present. This is the basis of the MHE technique.

To understand the principle behind MHE, let's first consider the situation in which the analyte is extracted from the vial in continuous process. We would see an exponential decay as shown in *Equation 26*.

$$M_t = M_1 \cdot e^{-q \cdot t} \quad \textbf{Equation 26}$$

Where:

- M_t is the mass of the compound in the vapor phase in the sample vial after time t
- M_1 is the mass of compound in the vapor phase in the vial before the extraction starts
- t is the elapsed time
- q is a constant dependent on several factors including extraction flow rate and vial size

If we apply *Equation 26* to a discontinuous extraction process such as MHE, this can be represented by *Equation 27*.

$$M_i = M_1 \cdot e^{-q \cdot (i-1)} \quad \textbf{Equation 27}$$

Where:

- M_i is the mass of compound in the vapor phase from the ith analysis
- i is the number of analyses performed

To calculate the total mass of the compound in the sample we assume that the analysis has been run repeatedly until no compound is left in the vial (i.e. i = infinity) and then we summate all the results for M_i obtained. This is shown mathematically in *Equation 28*.

$$M_{total} = \sum_{i=1}^{i=\infty} M_1 \cdot e^{-q \cdot (i-1)} \quad \textbf{Equation 28}$$

Where:

- M_{total} is the total extractable mass of compound from the sample. This is normally assumed to be the same as the mass originally in the sample.

Equation 28 is essentially a converging geometric progression which can be represented by *Equation 29*.

$$M_{total} = \frac{M_1}{(1-e^{-q})} \quad \textbf{Equation 29}$$

Thus, to calculate the total mass of compound, we only need to know, M_1 the mass of compound in the vapor phase at the start of the analysis and the value of the constant ***q.***

Because the volume of the vapor does not change during the sequence of repeat analyses, then the concentration of the compound in the vapor will be proportional to the mass present. The GC detector response will be proportional to the compound concentration and hence its mass. We can therefore substitute peak area in *Equation 27* as shown in *Equation 30*.

$$A_i = A_1 \cdot e^{-q\cdot(i-1)} \quad \text{Equation 30}$$

Where:

A_i is the GC peak area from the ith analysis

A_1 is the GC peak area from the first analysis

By taking the natural logarithm of both sides of *Equation 30*, we get *Equation 31*.

$$ln(A_i) = -q \cdot (i-1) + ln(A_1) \quad \text{Equation 31}$$

The value of ***q*** may be easily calculated by running a few MHE cycles and calculating the slope of the line for $ln(A_i)$ versus $-(i-1)$ from a graphic plot or from linear regression analysis. *Figure 18* shows an example application of MHE where instant coffee was analyzed for t-1,2-dichloroethylene. A small amount of water was added to the sample to improve the extraction efficiency. This plot shows a good linear relationship between the logarithm of the chromatographic peak areas and the extraction number. The value of ***q*** is determined to be 0.1028 in this instance (the negative of the slope) and hence the total amount of compound present may now be determined using *Equation 29*.

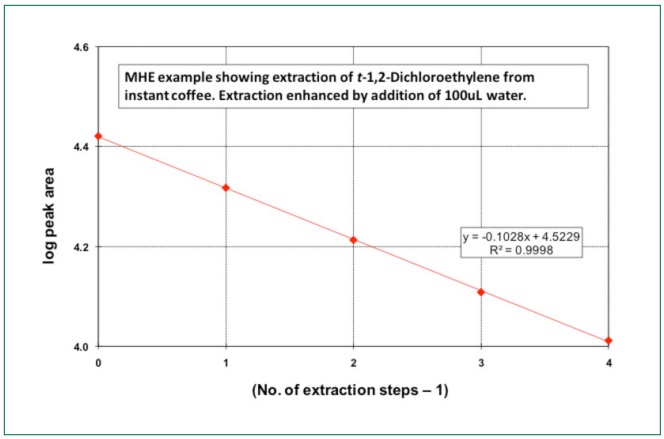


Figure 18. Example of an MHE analysis.

Once the value of ***q*** is known and the mass of compound in the first analysis, M_1 is established through calibration, the total mass of compound in the sample may be calculated using *Equation 29*.

MHE analysis still involves multiple extractions from the same sample vial but in most cases this may be limited to between 3 and 5 runs – a number much less than infinity.

Transferring the Headspace Vapor to the GC Column

So far we have limited the discussion to the processes occurring within the headspace vial. The next topic we should consider is the transfer of a representative sample of the headspace vapor to the gas chromatograph and into the GC column for analysis.

Injection Time and Volume

A typical HS sampling method will use a headspace vapor volume in the range of 10 to 20 mL. Modern high-resolution capillary columns may have carrier gas flow rates through them of 1.0 mL/min or even less. Injection of the whole headspace vapor into such columns may, therefore, take over 20 minutes – not a recipe for high-resolution gas chromatography!

In some instances, it may be possible to rely on the column to refocus the analytes at the inlet and still get acceptable chromatography but, for most applications, because the injected analytes are normally very volatile, there will be minimal on-column focusing. Therefore, we must reduce the injected volume of headspace vapor to a level that will reduce peak widths to provide acceptable chromatography on the column.

Figure 19 shows the effect on chromatography of increasing the injection volume of headspace vapor on to a medium resolution capillary column. These overlaid chromatograms of a single peak were produced on a TurboMatrix HS system that used pressure balanced sampling. This sampling technique allows direct control of the time width of the vapor plug entering the GC column. Under these conditions, it appears that the optimum plug width is around 0.03 minutes. Anything greater than this does not increase the peak height (or hence improve the detection limit) but does increase the peak width and hence reduce the ability of the column to resolve peaks.

The peak widths at the detector would normally be greater than the plug widths at the column inlet because of dispersion within the column. In this case they are not. Careful inspection of *Figure 19* shows that the peak widths are very close to the injected plug widths (annotated in the figure). This indicates that there is an element of focusing of the ethanol at the front of this column with a moderately thick stationary phase film.

In practice, the inlet plug width should be controlled directly or by adjusting the injection volume to be no greater than the peak width of the first eluting analyte.

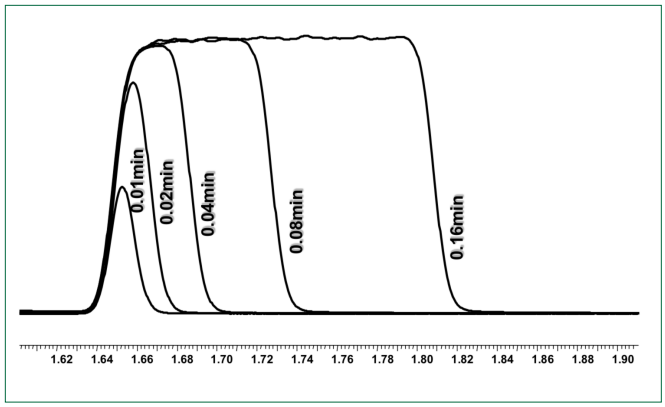


Figure 19. Effect of increasing the volume of injected headspace vapor into a GC column. 0.5% ethanol in water on 30 m x 0.3 2 mm x 1.0 µm PE-5 at 60 °C, helium at 12.5 psig (2.8 mL/min).

Note that in this discussion, we are talking about vapor plug widths and not vapor volumes. Although, in gas chromatography, we are used to describing injection amounts in terms of volume, in this situation we are concerned with keeping peak widths to an acceptable maximum– in HS sampling we do this by controlling the width of the sample plug entering the column.

Of course, the terms injected vapor plug width and volume are easily interchanged using *Equation 32* if the flow rate of carrier gas into the column is known.

$$V_{inj} = F_c \cdot t_{inj} \quad \textbf{Equation 32}$$

Where:

- V_{inj} is volume of vapor injected (normally adjusted to ambient temperature and pressure)
- F_c is the volumetric flow rate of carrier gas through the GC column (normally adjusted to ambient temperature and pressure)
- t_{inj} is the injected vapor plug width (same as the injection time in TurboMatrix methods)

For the example shown in *Figure 19*, with a plug width of 0.03 min, the injection volume would be calculated to be 2.8 x 0.03 = 0.084 mL. With a 20 mL headspace volume, this means that only 0.42% of the total headspace vapor actually enters the column, the other 99.58% is either left in the vial or is vented via a purging or splitting device.

With narrower-bore columns (e.g. 0.250 mm or 0.180 mm i.d.) the injected volume gets even less – perhaps even down to 0.02 mL.

There are various ways of taking headspace vapor out of a sample vial and injecting just a small fraction of it into a GC column. Some of these allow direct injection of undiluted headspace vapor into the column, others will use intermediate vessels that may cause dilution and splitters to produce narrow plug widths. Each of these has its own set of advantages and trade-offs. We will consider the various options in the following sections.

Manual Syringe Injection

The simplest way of taking a small volume of headspace vapor and injecting it into a GC is to use a manually operated gas-tight syringe. The sample is sealed in a vial in the normal way and then equilibrated thermally in an oven or water-bath. A gas syringe is used to withdraw a small volume of vapor from the vial (e.g. 1 mL) and inject it into a standard GC split inlet. Splitting is required to get sharp peaks.

While this technique seems inexpensive, simple and straightforward, it does suffer from a number of strong disadvantages:

- The vial may not be uniformly heated
- The temperature control of the vial may not be precise
- To take a sample involves manual access to the vial and may change its temperature
- It's difficult to maintain a uniform equilibration time for multiple vials
- Manual operation of the syringe will lack the precision achieved by instrumental control
- Vapor will be lost from the syringe when it is withdrawn from the vial
- The syringe will usually be at a lower temperature than the vial and so the risk of sample condensation is high
- Carrier gas will enter the syringe when it's inserted into the injector

At best, manual injection should be seen as a screening tool. It will not approach the analytical precision obtained using modern instrumentation.

Automated Gas Syringe Injection

Some headspace instruments adopt a gas syringe as a basis for transferring headspace vapor from a thermally equilibrated vial to a GC injector. This overcomes many of the concerns listed for manual gas syringe injection.

A heated syringe draws up a set volume of sample (typically 1 to 5mL). Because the syringe is normally at a higher temperature than the sample vial and the vapor inside the vial will be at higher pressure (see previous section on Vial Pressurization), there will be an expansion of the vapor and loss through the needle when the syringe needle is withdrawn from the vial. Thus the actual volume of headspace vapor extracted and remaining in the syringe will be less than the syringe volume set in the method as shown in *Equation 33*.

$$V_{Extract} = V_{Syringe} \cdot \frac{P_{Ambient}}{P_{Vial}} \cdot \frac{T_{Vial}}{T_{Syringe}} \quad \text{..... Equation 33}$$

Where:

$V_{Extract}$ is the volume of headspace vapor retained in the syringe after it is removed from the sample vial

$V_{Syringe}$ is the sampling volume set in the method

P_{Vial} is the absolute pressure of the headspace vapor inside the vial

$P_{Ambient}$ is the absolute ambient pressure

T_{Vial} is the absolute temperature of the headspace vapor inside the vial

$T_{Syringe}$ is the absolute temperature of the syringe

As an example, if the sample vial is held at 60 °C with an internal pressure of 5 psig and 1-mL headspace vapor is withdrawn into a syringe at 75 °C, then the actual volume of vapor collected in the syringe from the sample vial (at the pressure inside the vial) is:

$$1 \cdot \frac{15}{(5+15)} \cdot \frac{(60+273)}{(75+273)} = 0.72 \text{ mL}$$

For this calculation, the ambient pressure was assumed to be 15 psi.

The syringe then injects the extracted vapor into a split injector to which the column is connected. The effective volume of headspace vapor injected into the column is as shown in *Equation 34*.

$$V_{Sampled} = V_{Extract} \cdot \frac{F_{Column}}{(F_{Column}+F_{Split})} \quad \text{..... Equation 34}$$

Where:

$V_{Sampled}$ is the equivalent volume of headspace vapor actually injected into the GC column

F_{Column} is the flow rate of carrier gas through the GC column measured at ambient temperature and pressure

F_{Split} is the flow rate of carrier gas from the interface split vent measured at ambient temperature and pressure

Continuing with the example above, with an interface split flow rate of 25 mL/min and a column flow rate of 1 mL/min, the amount of headspace vapor that actually gets chromatographed will be:

$$0.72 \cdot \frac{1}{(1+25)} = 0.028\ mL$$

As is clearly seen here, the syringe volume setting in the method is not a good indication as to how much of the original headspace vapor is injected into the GC column – there are many other factors in this two-step injection technique that affect this.

Another important aspect of the injection process is how wide the plug of injected vapor is at the column inlet – this will have a direct effect on peak width and hence chromatographic performance.

The sample plug width will be depend on the volume of headspace vapor contained in the syringe and the total flow rate of gas meeting the column inlet as shown in *Equation 35*.

$$t_{Inject} = \frac{V_{Syringe}}{(F_{Column}+F_{Split})} \cdot \frac{P_{Inject}}{P_{Ambient}} \quad \text{..... Equation 35}$$

Where:

t_{Inject} is the width in time of the plug of vapor injected into the GC column

$V_{Syringe}$ is the volume of vapor inside the syringe

F_{Column} is the flow rate of carrier gas through the column measured at ambient temperature and pressure

F_{Split} is the flow rate of carrier gas from the interface split vent measured at ambient temperature and pressure

p_{Inject} is the absolute pressure inside the GC injector

$p_{Ambient}$ is the absolute ambient pressure

For example, with a 1mL syringe volume, 1mL/min column flow, 25 mL/min split flow and 15 psig injector pressure, the injection plug width would be:

$$\frac{1*60}{(1+25)} \cdot \frac{15+15}{15} = 4.6\ seconds$$

Although a 4.6 second injection plug width may seem significant, in many instances there may be some refocusing of the components on the GC column that would help achieve narrower and sharper peaks.

Valve Loop Injection

With valve loop injection, once the equilibration time is complete, a sampling needle is inserted through vial septum and the sample vial is pressurized to provide a final pressure of 1.5 to 2.0 atmospheres (22.5 to 30 psig). The pressurized vapor is then allowed to escape through a valve sampling loop and out to vent. In many respects this step is very similar to the pressure balanced sampling technique described later except that instead of being diverted directly into the GC column or transfer line, the vapor is held in the sampling loop.

The sampling loop has a fixed capacity which is normally 1 mL. It is held at a temperature typically 15 °C above that of the sample vial to prevent sample condensation. The pressure in the charged loop will be less than that inside the vial and will normally be at ambient pressure at the end of the sampling process. It is possible to connect some restrictor tubing to the loop vent and terminate the sampling early to leave a higher residual sample pressure inside the loop to increase sensitivity.

It is important at this point to appreciate how much of the headspace sample vapor is actually held in the loop at this point as shown in *Equation 36*.

$$V_{Extract} = V_{Loop} \cdot \frac{P_{Loop}}{P_{Vial}} \cdot \frac{T_{Vial}}{T_{Loop}} \quad \text{..... Equation 36}$$

Where:

- $V_{Extract}$ is the volume of headspace vapor removed from the sample vial
- V_{Loop} is the valve sampling loop capacity
- P_{Vial} is the absolute pressure of the headspace vapor inside the vial
- P_{Loop} is the absolute pressure inside the valve sampling loop at the end of the sampling step
- T_{Vial} is the absolute temperature of the headspace vapor inside the vial
- T_{Loop} is the absolute temperature inside the valve sampling loop at the end of the sampling step

As an example, if the sample vial is held at 60 °C with an internal pressure of 20 psig and a 1 mL valve loop is charged with headspace vapor at 75 °C at a final pressure equivalent to ambient, then the actual volume of vapor collected in the loop from the sample vial (at the pressure inside the vial) is:

$$1 \cdot \frac{15}{(20+15)} \cdot \frac{(60+273)}{(75+273)} = 0.41 \text{ mL}$$

For this calculation, the ambient pressure was assumed to be 15 psi.

Note that if the sampling period is terminated early to charge the loop with headspace at a higher pressure to increase the amount sampled, then the actual amount of sample vapor in the loop would be higher but essentially unknown. A pressure gauge could be plumbed into the system to enable the loop pressure to be monitored and so allow the amount of vapor sampled to be calculated.

After sampling, the valve rotates and a manual flow controller supplies a fixed flow rate of carrier gas into the loop and into a transfer line connected to the GC.

A split interface at the GC supplies further carrier gas which mixes with the sample flow from the headspace system and a large fraction of the total combined flow rate is passed to a split vent. Splitting is necessary to achieve peaks that would be sharp enough for narrow-bore capillary columns. A 1:1 split is suggested but many methods require higher split ratios such as 50:1.

This splitting further reduces the amount of sample vapor reaching the GC column according to *Equation 37*.

$$V_{Sampled} = V_{Extract} \cdot \frac{F_{Column}}{(F_{GC}+F_{Loop})} \quad \text{..... Equation 37}$$

Where:

- $V_{Sampled}$ is the equivalent volume of headspace vapor actually injected into the GC column
- F_{Column} is the flow rate of carrier gas through the GC column measured at ambient temperature and pressure
- F_{Loop} is the flow rate of carrier gas set on the HS mass flow controller to deliver the contents of the loop to the GC
- F_{GC} is the flow rate of carrier gas added for splitting from the GC measured at ambient temperature and pressure. This is not the flow measured at the split vent.

Continuing with the example above, with a loop flow of 10 mL/min, a GC flow rate of 25 mL/min and a column flow rate of 1 mL/min, amount of headspace vapor that actually gets chromatographed will be:

$$0.41 \cdot \frac{1}{(10+25)} = 0.012 \text{ mL}$$

This result is clearly very different from the 1 mL capacity of the valve loop being used.

The valve loop injection is different from the syringe injection in that the contents of the loop are transferred to the GC injector by a controlled flow of carrier gas. Thus it will take a measurable time for the whole sample vapor to enter the injector liner.

The vapor width at the column inlet is described by *Equation 38*.

$$t_{Inject} = \frac{V_{Loop}}{F_{Loop}} \cdot \frac{P_{Injector}}{P_{Ambient}} \cdot \frac{T_{Ambient}}{T_{Injector}} \quad \text{..... Equation 38}$$

The capacity of the loop itself is not indicative of how much sample is actually injected. Pressure and temperature changes and applied splits all have a direct effect on the volume of headspace vapor directed to the GC column. Out of the three types of HS systems, this is the most difficult to predict how much of the vapor actually gets injected. Unless all the parameters are well understood and measurable, it is impossible to determine how much sample is actually injected with a valve loop injection system.

Pressure Balanced Sampling

In practice, pressure balanced sampling actually provides the most straightforward means of determining the amount of headspace vapor injected.

Pressure balanced sampling has the very big advantage in that it is a single-stage injection technique in which sample vapor from the headspace vial flows directly into the GC column. Methods can be set up in which the sample stream is not subjected to dilutions or losses in the transfer process. All the critical parameters are straightforward to determine when calculating the injection volume and it is easy to adjust this volume as it is directly proportional to the sampling time entered in the method.

There are three ways the vapor can be transferred from the vial into the GC column in pressure balanced sampling:

Direct Connection

In this mode, the column is connected directly to the headspace sampling tower. This means that the headspace vapor exits the pressurized sample vial and passes directly into the GC column with no dilution with carrier gas or change in pressure. Because the time-width of the vapor plug is precisely controlled, if the flow rate into the column is known, the volume of the headspace vapor injected is easy to calculate as shown in *Equation 39*.

$$V_{Sampled} = F_{Column} \cdot \frac{P_{Ambient}}{P_{Vial}} \cdot \frac{T_{Vial}}{T_{Ambient}} \cdot t_{Inject} \quad \text{..... Equation 39}$$

Where:

- $V_{Sampled}$ is the equivalent volume of headspace vapor at the pressure and temperature inside the sample vial actually injected into the GC column
- F_{Column} is the flow rate of carrier gas at the outlet of the GC column measured at ambient temperature and pressure
- $P_{Ambient}$ is the absolute ambient pressure under which FColumn was measured
- P_{Vial} is the absolute ambient pressure of the headspace vapor inside the vial
- $T_{Ambient}$ is the absolute temperature under which FColumn was measured
- T_{Vial} is the absolute temperature of the headspace vapor inside the vial
- t_{Inject} is the injection time set in the method

For example, with a vial pressure of 18 psig and temperature of 60 °C, a column flow rate of 1mL/min and a 0.04 min sampling time gives an effective sampling volume of:

$$1 \cdot \frac{15}{(18+15)} \cdot \frac{(60+273)}{(23+273)} \cdot 0.04 = 0.020 \text{ mL}$$

For this calculation, $P_{Ambient}$ was assumed to be 15 psi and $T_{Ambient}$ was assumed to be 23 °C.

Some users prefer to use a length of narrow-bore fused silica tubing butt-connected between the column and the headspace sampler to allow quick exchange of the column. The performance is not significantly affected by the presence of this transfer line.

It is also important to establish any dilution of the sample vapor by carrier gas during the transfer and the consequential effect on the width of the injection and effective volume at ambient temperature and pressure actually injected into the column. This information will provide guidance on the 'efficiency' of the transfer and the likely effect on chromatographic peak widths.

In the case of the direct injection mode, there is no effective dilution and so the injection width will be the same as the injection time set in the method. The dilution will be zero and the effective injection volume may be calculated as shown in *Equation 40* where V_{injAmb} is the injection volume corrected for ambient temperature and pressure.

$$V_{InjAmb} = F_{Column} \cdot t_{Inject} \quad \text{Equation 40}$$

Because the sampling time is easy to control, systems that use this approach are able to easily change the injection volume over a large range (e.g. > 20:1) by a simple method adjustment with no changes to the hardware.

Split Injector Interface

In this interfacing technique, a length of narrow-bore fused silica is connected between the headspace sampler and a split injector on the GC. The vial pressure is set higher than the injector pressure so that the headspace vapor will flow into the injector liner at about 25 mL/min. Some of the vapor will enter the column and the rest will exit from the injector split vent. The carrier gas controller on the GC will control the pressure across the column and so some flow of carrier gas will enter the injector and mix and dilute the headspace vapor before it enters the column. Note that the splitter in this case does not directly affect the volume of sample vapor entering the GC column. The volume of vapor injected will be dependent on the flow rate into the column and the injection time set on the headspace system and not on the split flow rate.

Even though the flow rate from the sample vial during sampling is much greater than the flow rate of vapor entering the column and the pressure and temperature inside the injector are normally different from those inside the sample vial, these terms cancel out and *Equation 39* will still apply. What must be taken into account, however, is the dilution effect of adding additional carrier gas into the injector liner for chromatographic carrier gas control. This is taken in to account in *Equation 41*.

$$V_{Sampled} = F_{Column} \cdot \frac{F_{Vial}}{(F_{Vial}+F_{GC})} \cdot \frac{P_{Ambient}}{P_{Vial}} \cdot \frac{T_{Vial}}{T_{Ambient}} \cdot t_{Inject} \quad \text{Equation 41}$$

Where:

$V_{Sampled}$ is the equivalent volume of headspace vapor at the pressure and temperature inside the sample vial actually injected into the GC column

F_{Column} is the flow rate of carrier gas at the outlet of the GC column measured at ambient temperature and pressure

F_{Vial} is the flow rate of sample vapor from the sample vial measured at ambient temperature and pressure

F_{GC} is the flow rate of the additional carrier gas added by the GC controller measured at ambient temperature and pressure

$P_{Ambient}$ is the absolute ambient pressure under which FColumn was measured

P_{Vial} is the absolute ambient pressure of the headspace vapor inside the vial

$T_{Ambient}$ is the absolute temperature under which FColumn was measured

T_{Vial} is the absolute temperature of the headspace vapor inside the vial

t_{Inject} is the injection time set in the method

The flow rate of carrier gas added by the GC can be very low or even off. The PerkinElmer gas chromatographs are able to operate in a 'headspace' mode in which carrier gas from the HS system is pressure regulated at the GC without the need to supply additional gas from the GC. Its main function is to provide control of the carrier gas through the GC and to flush the internal plumbing lines so that headspace vapor does not diffuse into unswept lines and cause cross-contamination issues or ghost peaks. Typically 5 mL/min is sufficient.

Applying the GC flow rate to the earlier example, gives:

$$1 \cdot \frac{25}{(25+5)} \cdot \frac{(15)}{(18+15)} \cdot \frac{(60+273)}{(23+273)} \cdot 0.04 = 0.017\ mL$$

With a split injector interface, there is going to be some dilution of the headspace vapor as it is transferred to the GC column. In the first instance, it will enter a liner of finite volume and so will disperse within this space. Secondly it will mix with carrier gas from the GC and be diluted as a result.

In practice with a liner capacity of 200 µL or less and a flow rate through it in excess of 15 mL/min, these will be little effect on the width of the sample vapor plug entering the GC column. This will remain the same as that entered in the method and the injection volume calculation will use the same equation as for direct injection.

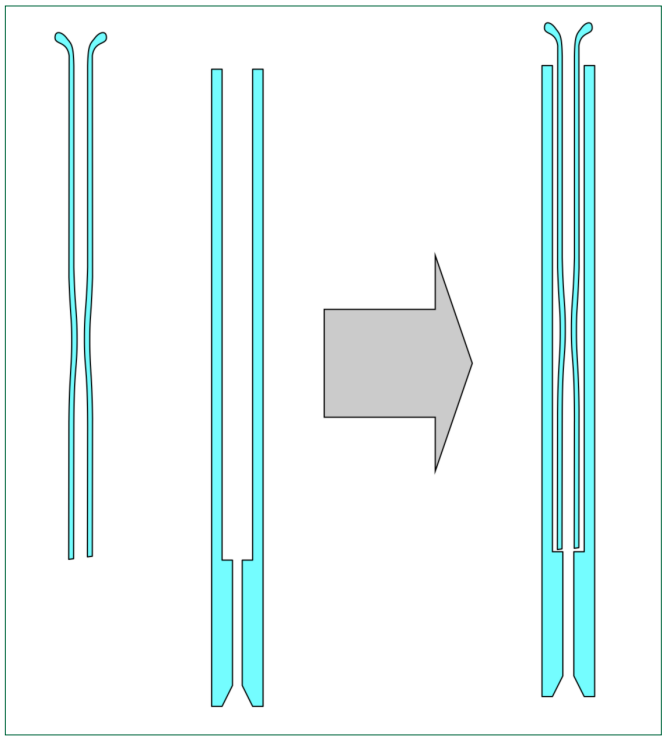


Figure 20. Schematic diagram showing inner and outer components of the ZDL.

Split Injector Interface with Zero Dilution Liner (ZDL)

This is identical to the previous mode except that a special patented injector liner is used to prevent the carrier gas from the GC from mixing with the headspace vapor and so totally eliminates the dilution effects on the sample vapor as it passes through the injector and enters the GC column. Equation 39 is, therefore, directly applicable to this mode of injection.

Figure 20 and *Figure 21* illustrate the working principle of the ZDL. There is an inner and an outer component of this liner. The gas stream from the HS system enters the inner liner from the transfer line and immediately enters the GC column. The gap between the transfer line and the column is only a few mm and is totally inert so the integrity of the sample vapor should be unaffected. Excess flow of gas from the transfer line exits the top of the inner liner and excludes the GC carrier gas entering the injector from reaching the column. The GC carrier gas is restricted to the outer liner and serves to keep the system clean and to maintain control of the carrier gas pressure applied to the column inlet.

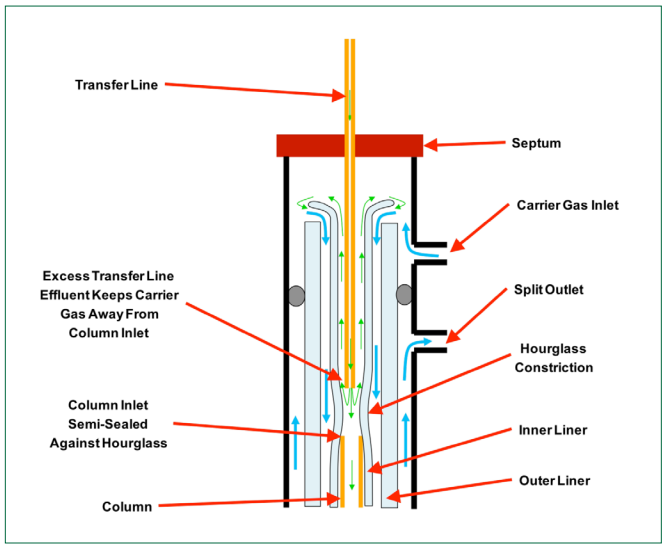


Figure 21. Schematic diagram showing the installed ZDL inside a split injector. Note how the excess flow from the sample stream entering the system from the transfer line prevents the GC carrier gas from entering the column.

Figure 22 shows the result of using a ZDL for a typical HS application. Increased response is observed (up to 6x improvement in this case) which is also unaffected by changing the GC split flow rate.

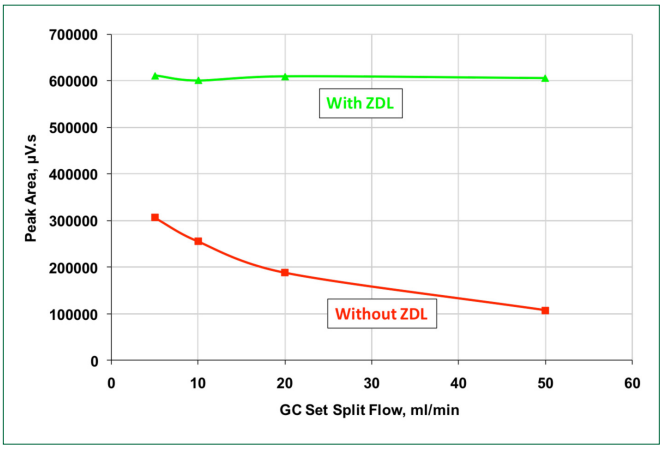


Figure 22. Chart illustrating how the use of a ZDL eliminates the dilution effect occurring inside a GC injector as a result of splitting. These data were produced on a TurboMatrix HS system on water samples containing ethanol.

Improving Detection Limits

Equilibrium headspace sampling is a very effective way of extracting and injecting volatile compounds from difficult sample matrices. However only a fraction of the analytes will partition into the vapor phase inside the vial (dependent on the partition coefficient, of course) and only a very small fraction of the total vapor will be actually introduced into the GC column.

For instance, the injected vapor volume may be as low as 0.01 mL at the pressure inside the vial. If the total headspace volume is 10 mL, then only 0.1% of it will be injected into the GC column and 99.9% of it will remain in the vial or will be vented by splitting.

Injecting a greater volume of headspace vapor will generally simply increase the peak width without improving detection limits as shown in the example given in earlier in *Figure 19*. The TVT and FET techniques will provide some enhancements to detection limits for certain types of sample. However, what is really needed is some way of introducing more of the headspace vapor into the GC column.

One way of achieving this is the use of some form of intermediate trapping device to extract and focus the analytes and then release them as a narrow plug of vapor into the GC column.

There are various ways in which this focusing and re-vaporization can be achieved as described in the following sections.

Sample Stacking On Column

This is a technique offered by some systems in which multiple headspace vapor extractions are taken from a single vial or single extractions are taken from multiple vials of the same sample and injected into the GC column where they are retained. In this situation the column is used as the trapping device. The GC oven is programmed to produce chromatography at the end of the final extraction. While this simple technique may give bigger peaks the majority of the vapor will still not be injected. The total amount injected using this approach is given by *Equation 42*.

$$M_{Stacked} = M_{Single} \cdot n \quad \text{.....Equation 42}$$

Where:

$M_{Stacked}$ is the mass of analyte stacked on the column prior to chromatography

M_{Single} is the mass of analyte transferred to the column from a single extraction (assumed to be the same for each extraction)

n is the total number of extractions

In the earlier example where only 0.1% of the total headspace vapor was injected into the GC column, after 10 extractions with column stacking, still only 1% of the analytes would be introduced into the GC column.

The technique will also be very time consuming and relies on the ability of the column to retain the analytes from the injected vapors until the final extract is injected. In many applications this will not work because the volatile analytes are not sufficiently retained on the column.

Alternative techniques to concentrate the whole or most of the headspace vapor are generally preferred.

On-Column Cryofocusing

One technique that was successfully used for many years was to cool the GC column and so re-focus the compounds in a higher volume of injected vapor. By temperature programming the column once the injection was complete, peaks eluted that were narrow and higher thus retaining chromatographic resolution and significantly improving detection limits.

The simplest means of achieving on-column re-focusing, would be to deploy a sub-ambient accessory for the GC oven that would use liquid nitrogen or liquid carbon dioxide to cool the whole oven.

In practice, whole oven cooling is rather overkill. We don't want to perform chromatography at these temperatures but rather just focus the compounds in a few milliliters of vapor at the column inlet. A practical approach to on-column re-focusing is shown in *Figure 23*. The first loop of the GC column is threaded through a length of thin-walled PTFE tubing through which a stream of cooled nitrogen gas is flowing. This creates a very effective cooling sheath around this section of the column and is able to re-focus almost all organic compounds at the column inlet. The nitrogen gas is cooled by passing it through a heat exchanger made from a coil of copper tubing which is submerged in liquid nitrogen held inside an insulated Dewar flask. The flow of gas is turned on and off by a solenoid valve under the control of the GC method. Using cooled gas in this way enables the cooling process to be rapidly applied and when turned off, the GC column quickly returns to the oven temperature. Cooling temperatures of -150 °C or even below are easily achieved.

The relationship between sampling time and amount injected will remain the same for regular pressure balanced sampling.

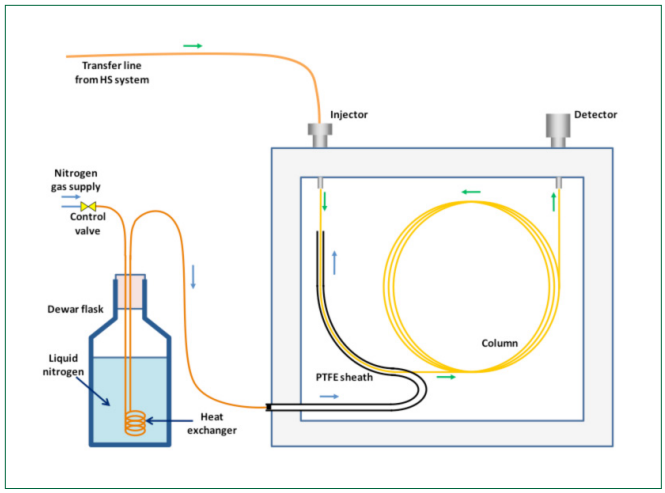


Figure 23. Practical system for on-column cryo-focusing of compounds from headspace vapor.

Figure 24 shows a chromatogram of a mixture of low-level halogenated hydrocarbons in water that was produced using a long (2 minutes) sampling time and with the on-column cryo-focusing system shown in *Figure 23*.

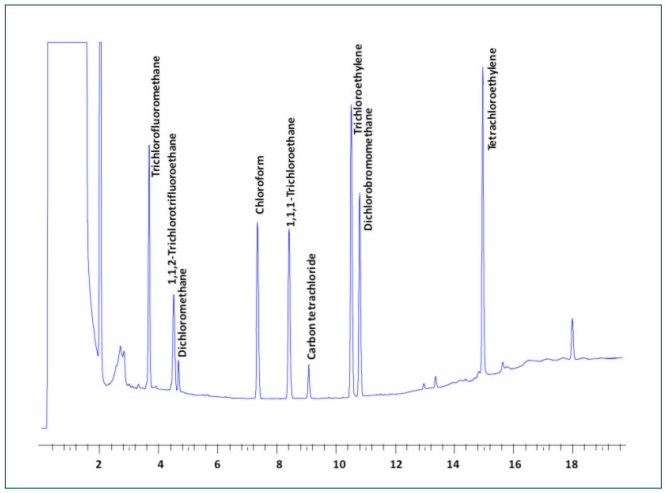


Figure 24. Electron capture chromatogram of 3 to 1000 ppt volatile halogenated hydrocarbons in water using 2-minute headspace sampling with on-column cryofocusing.

Note that although the system shown in *Figure 23* is very effective in improving detection limits, there are a few caveats that must be considered. The main issue is the presence of water in the headspace vapor. This water will condense and freeze inside the section of cooled column and will easily block the flow of vapor through it thus effectively ruining the analysis. To address this issue, a water abstractor device containing a desiccant such as lithium chloride or potassium carbonate may be inserted into the sample vapor stream. This removes the moisture at lower temperatures and is reactivated by the heat of the GC oven when temperature programmed.

Dynamic Headspace Sampling

Dynamic headspace is a technique very similar to equilibrium (static) headspace sampling but is intended to direct most, if not all, of the headspace vapor into the GC column. It is also very similar to the purge and trap technique except that the incoming gas supply is introduced into the headspace rather than made to bubble through the sample.

Two needles are used to puncture the vial seal: one to introduce carrier gas and the other to provide an outlet. Normally the two needles are combined into a concentric arrangement for mechanical simplicity. Some form of trap is located in the outlet path. *Figure 25* shows a schematic diagram of a typical dynamic headspace setup using a stream of carrier gas to drive the headspace vapor into a suitable trap. The trap normally comprises a series of adsorbent beds that will retain the analytes.

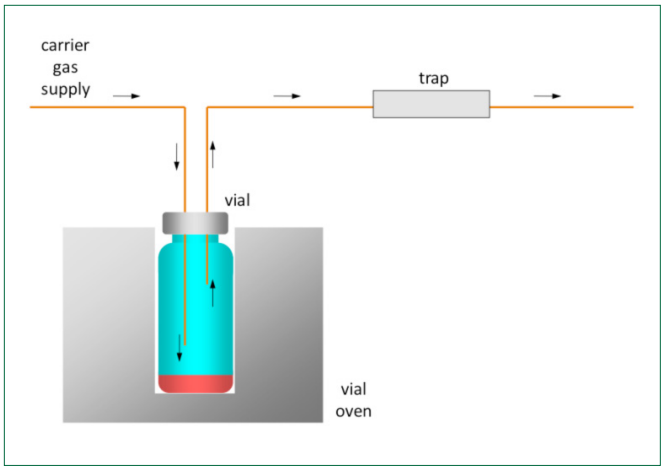


Figure 25. Schematic diagram of a typical dynamic headspace system showing the trap load step.

The concentration of compounds in the headspace phase will undergo exponential dilution as described by *Equation 43*.

$$C_t = C_0 \cdot e^{\frac{-F_p \cdot t}{V_G}} \quad \text{Equation 43}$$

Where:

- C_t is the concentration of a compound in the vial headspace after time, t
- C_0 is the initial concentration of a compound in the vial headspace before purging
- F_p is the carrier gas purge rate (at the pressure and temperature inside the vial)
- t is the purge time
- V_G is the volume of the headspace phase in the vial

Equation 43 may be re-arranged to *Equation 44*.

$$t = \frac{-V_G}{F_p} \cdot \ln\left(\frac{C_t}{C_0}\right) \quad \text{Equation 44}$$

Equation 44 enables the purge time to be calculated in order to remove, for instance, 99% (or leave 1%) of the sample vapors from the vial as shown in *Equation 45*.

$$t = \frac{-V_G}{F_p} \cdot \ln\left(\frac{1}{100}\right) \quad \text{Equation 45}$$

The sample is prepared and equilibrated in the same way as for regular equilibration headspace. The trapping device may be a cold spot in a tube or column or may be a purpose-designed adsorbent trap.

After the vial has been swept and the sample vapors have been collected in the trap, the trap is heated to vaporize the collected compounds and valve changes are made so that carrier gas carries the compounds into the GC and column for analysis as shown in *Figure 26*.

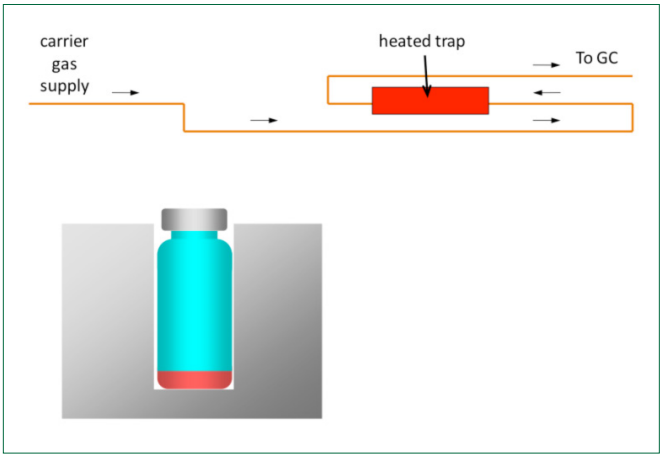


Figure 26. Schematic diagram of a typical dynamic headspace system showing the trap desorption step.

This technique is able to improve detection limits for analytes in samples by a factor of 100x or even more.

Headspace Trap Sampling

Headspace trap sampling uses equilibrium headspace to produce a stable headspace vapor. The sample vial is pressurized to a high pressure and then the pressure is allowed to decay by allowing the vapor to flow through an adsorbent trap to vent. In this way most of the headspace vapor may be extracted and the compounds in it are retained on the trap. Extraction will eventually stop once the pressure inside the vial is the same as ambient pressure; thus some vapor is left in the vial at the end of this process.

Figure 27 shows a schematic diagram of a HS Trap system in the trap load mode. In this instance, the vial has been thermally equilibrated and pressurized with carrier gas in the same way as for the standard pressure balanced sampling technique. The pressurized headspace vapor inside the vial is allowed to vent through an adsorbent trap which retains the analytes. An isolating flow of carrier gas keeps the headspace vapor out of the GC column during this step.

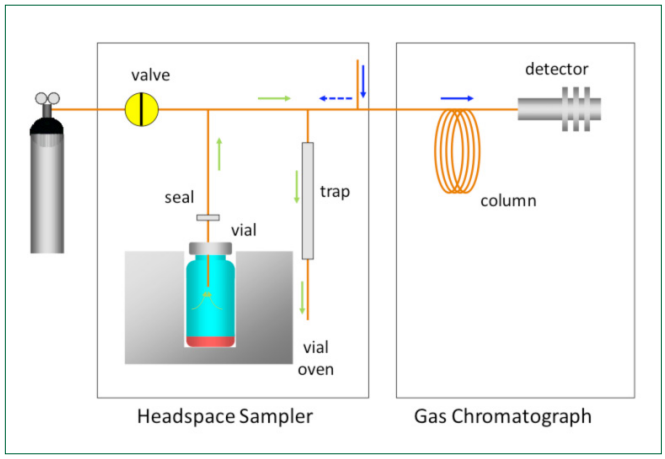


Figure 27. Schematic diagram showing HS Trap in trap load mode.

Once the analytes are in the trap, the isolating flow is turned off, the flow of carrier gas is reversed and the trap is heated as shown in *Figure 28*. The thermally desorbed analytes are carried by the carrier gas into the GC column where they are separated and detected.

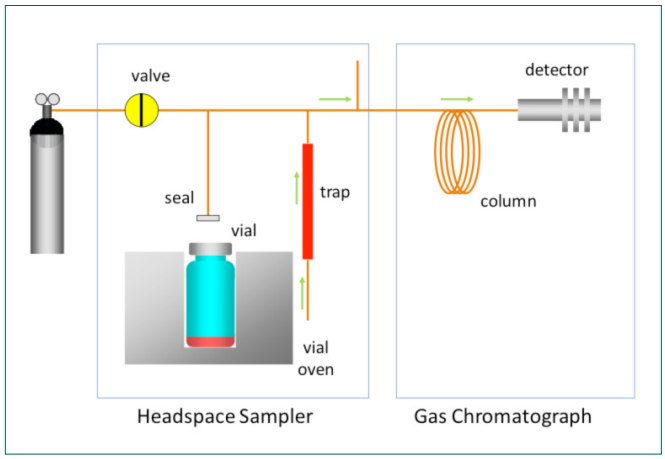


Figure 28. Schematic diagram showing HS Trap in trap desorption mode.

The act of pressurizing the sample vial and venting it through a trap will not extract the entire vapor from the vial – after venting, vapor is still left in the vial at atmospheric pressure. The percentage residue left in the vial after a single pressurization/venting cycle may be represented by *Equation 46*.

$$R = 100 \cdot \frac{p_{lo}}{p_{hi}} \quad \text{.....Equation 46}$$

Where:

R is the percent residue of the initial vapor left in the vial

p_{lo} is the absolute pressure after venting through the trap (normally atmospheric)

p_{hi} is the elevated absolute pressure inside the vial prior to venting

Note that the result is independent of the volume of the headspace and its temperature.

For example, for p_{hi} = 40 psig, one pressurization/venting cycle will leave about 23% of the original vapor in the vial and 77% would be passed into the trap. Increasing the value of p_{hi} or reducing the value of p_{lo} would increase the extraction but these may not not practical on standard instrumentation.

A better way to increase the extraction efficiency is to perform multiple pressurization/venting cycles. Each cycle would reduce the residual vapor in the vial. *Equation 47* shows the residue left after multiple cycles.

$$R = 100 \cdot \left(\frac{p_{lo}}{p_{hi}}\right)^{n} \quad \text{.....Equation 47}$$

Where:

n is the number of pressurization/venting cycles

Figure 29 shows how the number of pressurize/venting cycles affects the residual vapor left in the vial for a range of elevated pressures vented to atmospheric pressure.

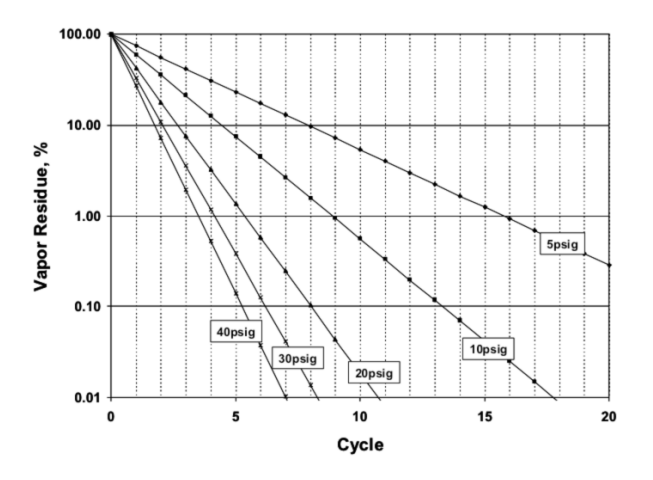


Figure 29. Theoretical effect of pressurization/venting cycles on residual vapor left in sample vial over a range of elevated pressures.

The information shown in *Figure 29* enables us to define the number of cycles necessary to get the vapor out of the vial. Again, note that this will be independent of the volume of the headspace and its temperature.

Table 5 shows the number of cycles necessary to get 99% and 99.9% of the headspace vapor out of the vial.

Table 5. Number of pressurization/venting cycles necessary to extract headspace vapor from sample vials.

	Cycles to Extract Percentage Vapor	
Pressure, psig	**99%**	**99.9%**
5	16	Too Many
10	9	13
20	5	8
30	4	6
40	4	5

As the pressure is decreased, then more cycles are needed to extract the whole headspace vapor. The data in *Table 5* may be represented by *Equation 48*.

$$n = Integer\ \left[\frac{\log\left(\frac{(100-R)}{100}\right)}{\log\left(\frac{Plo}{Phi}\right)} + 0.5 \right] \quad \text{Equation 48}$$

Equation 48 provides the means of calculating the number of pressurization/venting cycles, ***n,*** necessary to extract the headspace vapor out of the vial to leave a percent residue, ***R.***

Another aspect of HS Trap sampling that should be considered is the time it takes to vent the headspace vapor into a trap. If we use a simple restrictor to limit the flow rate of vapor passing into a trap, it can take a significant time to make this extraction as shown in the experimental pressure decay curves in *Figure 30*. If multiple extractions are going to be performed then the total extraction time is going to be prohibitively long. These exponential decay profiles occur because although the flow rate of vapor from the vial into the trap may be initially high, the flow rate drops as the pressure of the vapor inside the vial drops. To get the last 10% of vapor out of the vial may take a long time and even then the vial is still full of vapor when atmospheric pressure is finally reached. This is not an efficient way of getting the vapor out of the vial and into the trap.

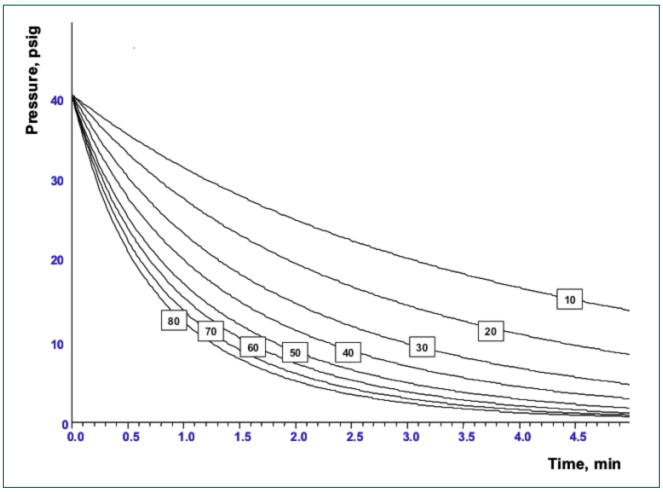


Figure 30. Experimental pressure decay profiles from a 22 mL vial, pressurized to 40 psig at 60 °C and vented through a restrictor at different initial flow rates (values in boxes on chart indicate initial flow rate in mL/min).

By substituting a simple flow controller instead of the restrictor, the flow rate of vapor from the vial into the trap is maintained through the pressure detail making the process much more efficient. The pressure decay profile is linear and the venting times become much shorter as shown in *Figure 31*.

Using this approach, even a vial with 22 mL of headspace vapor in it can be transferred to the trap within 1 minute.

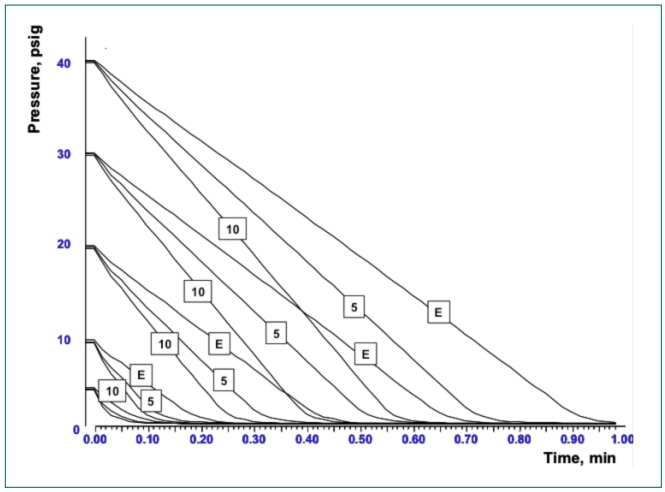


Figure 31. Experimental pressure decay profiles using a 50 mL/min constant flow device to regulate the extraction of vapor from a pressurized 22 mL HS sample vial at 60 °C. E = empty vial, 5 = vial with 5 mL water added, 10 = vial with 10 mL water added.

Note how the linear pressure decay profiles vary significantly with the amount of sample inside the vial. The slope of these pressure decay profiles can be used to measure the sample volume or confirm that it is correct inside the vial at the time of analysis. The pressure decay profiles will also be affected by any leaks in the vial seal. This information can be used as an additional confirmation that the analysis was leak-free and performed correctly.

Finally, we should consider the importance of sample volume in HS Trap sampling. In this situation our chromatographic peak sizes are going to be dependent on the total mass of compounds in the whole headspace vapor – because that's what is going to be collected in the trap. This is different from regular headspace where it is the concentration of an analyte that dictates the peak size.

The total mass of a compound in the headspace is the product of the headspace volume and the compound concentration in it as shown in *Equation 49*.

$$M_G = V_G \cdot C_G \quad \text{Equation 49}$$

Substituting *Equation 15* from the beginning of this document into *Equation 50* gives Equation

$$M_G = C_0 \cdot \frac{V_G}{K + \beta} \quad \text{Equation 50}$$

Figure 32 shows *Equation 50* applied to a 1µg/mL sample added to a 22 mL vial to see how total mass of compound in the headspace varies with sample volume and the value of K, the partition coefficient. Note the logarithmic scale for the y-axis.

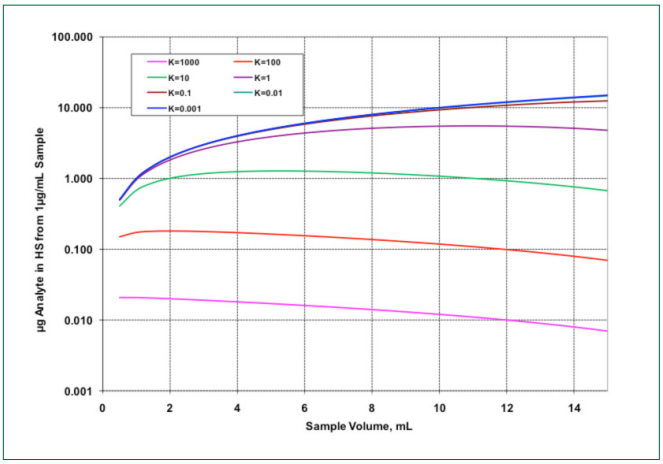


Figure 32. Total Amount of Analyte in the Headspace Vapor for Different Partition Coefficients (K) at Different Sample Volumes in a Standard 22 mL Headspace Vial.

What is perhaps counter-intuitive is that in all cases except when the value of ***K*** is less than about 1, the total mass of compound in the headspace phase decreases as more sample is added to the vial. This is not the case with standard (static) headspace – compare *Figure 32* with *Figure 7* to *Figure 9*. Thus in these cases, detection limits are significantly enhanced by using smaller samples.

For instance when analyzing water containing 1µg/mL ethanol at an equilibration temperature of 60°C when ***K*** has a value of 511, the expected mass of ethanol in the headspace phase would be 0.0395 µg with a sample volume of 0.5 mL yet it would only be 0.0195 µg with a 12 mL sample – this is half the effective sensitivity for twelve times the sample volume!

Note that as the headspace volume is increased, if the sample contains water, the weight of water vapor in the headspace is directly proportional to the headspace volume. Some trade-offs between better detection limits and increased water management will have to be made with some samples.

Solid Phase MicroExtraction (SPME)

SPME is another technique that can be used to extract and concentrate compounds from headspace vapor. Instead of using carrier gas to sweep or pulse the headspace vapor out of the sample vial into some sort of trapping device, SPME essentially inserts a 'trap' into the headspace vapor inside the vial. This 'trap' is normally implemented in the form of a retentive coating applied to a narrow fused silica fiber which is located within the needle of a special syringe as shown in *Figure 33*. This syringe is normally operated by an autosampler but the whole process may be performed manually if required. The needle pierces the seal of a vial containing the sample and the coated fiber extends down into the headspace and starts to absorb or adsorb compounds from the vapor. The system is left to stabilize or equilibrate for a period of time. The fiber is drawn back into the syringe needle which itself is withdrawn from the vial and inserted into a heated GC inlet. The fiber is extended and absorbs heat from the injector liner which desorbs the extracted analytes and carrier gas transfers them to the GC column for analysis.

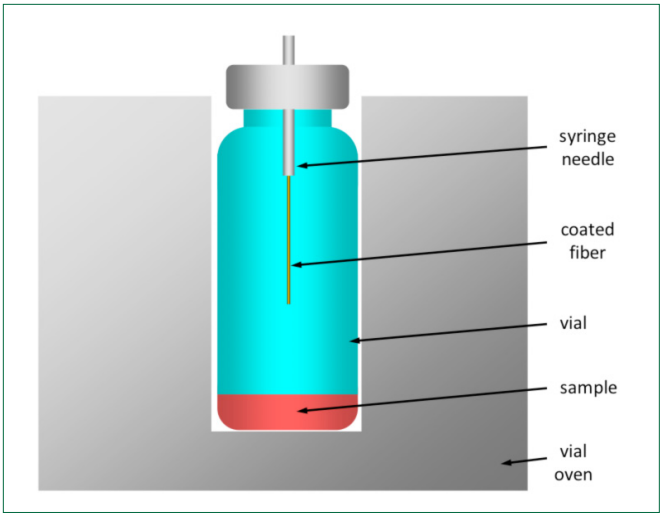


Figure 33. Schematic diagram showing principle of SPME headspace sampling.

SPME considerably simplifies the extraction technique – no gases or plumbing are required. It provides a good degree of analyte pre-concentration for many analytes and is very effective at eliminating the effects of water, etc. which may enter the trap and column with other techniques.

However in terms of the theory behind the extraction process, things are significantly more complex. It is beyond the scope of this document to delve very deeply into the theory of SPME and the reader is recommended to refer to excellent books that are available on this topic [6, 7 and 8].

Essentially, HS sampling by SPME is a 3-phase system. The headspace vapor phase will interact with the sample phase and with the SPME fiber coating. Two thermodynamic systems are at work simultaneously: analytes will seek to achieve an equilibration between the sample and the headspace vapor with a concurrent equilibration taking place between the headspace vapor and the fiber coating. Thus two partition coefficients are involved to achieve a final equilibration in the system.

To understand the relationships involved, let's start with the mass balance equation shown in *Equation 51*. The mass of a compound in each phase will be the product of its concentration in that phase and the volume in that phase. This equation simply shows that the masses in the phases when added together will equal the mass in the original sample added to the vial.

$$C_0 \cdot V_S = C_F \cdot V_F + C_S \cdot V_S + C_G \cdot V_G \quad \text{.....Equation 51}$$

Where:

C_0 is the concentration of the compound in the sample added to the vial

C_F is the concentration of the compound in the fiber coating at equilibrium

C_S is the concentration of the compound in the sample (liquid) phase at equilibrium

C_G is the concentration of the compound in the headspace (gas) phase at equilibrium

V_F is the volume of the fiber coating

V_S is the volume of the sample added to the vial

V_G is the volume of the headspace (gas) phase

Note that different texts seem invert these definitions – in this case we wanted to preserve the conventions already used in this document.

$$K_{FG} = \frac{C_F}{C_G} \quad \text{.....Equation 52}$$

$$K_{SG} = \frac{C_S}{C_G} \quad \text{.....Equation 53}$$

Where:

K_{FG} is the partition coefficient between the fiber coating and the headspace phase

K_{SG} is the partition coefficient between the sample phase and the headspace phase

Note that *Equation 53* is equivalent to *Equation 1* provided at the start of this document.

For informational purposes, even though the fiber coating does not make contact with the sample phase in headspace sampling, the partition coefficient of a compound between these phases can derived from *Equations 52* and *53* as shown in *Equation 54.*

$$K_{FS} = \frac{C_F}{C_S} = \frac{K_{FG}}{K_{SG}} \quad \text{Equation 54}$$

Where:

K_{FS} is the partition coefficient between the fiber coating and the sample phase

An important implication of *Equation 54* is that it doesn't really matter if the fiber makes contact with the headspace, the sample or both – the amount of compound extracted will (in theory) be the same.

Again, different texts will use different conventions for defining partition coefficients. The definition in *Equation 54* indicates that components with high values will favor the fiber coating whereas those with low values will favor the sample phase.

We can rearrange *Equation 51* to include the partition coefficients defined in *Equations 52* and *53* to give *Equation 55*.

$$M_F = C_0 \cdot \frac{K_{FG} \cdot V_F \cdot V_S}{(K_{FG} \cdot V_F + V_G + K_{SG} \cdot V_S)} \quad \text{Equation 55}$$

Where:

M_F is the mass of analyte extracted into the fiber coating at equilibrium

Because the value of VF is so small, *Equation 55* may be rearranged to give *Equation 56*.

$$M_F = C_0 \cdot \frac{K_{FG} \cdot V_F}{[K_{SG} + \beta_{SG}]} \quad \text{Equation 56}$$

Where:

β_{SG} is the ratio of the volumes of the two phases (VG/VS)

β_{SG} is equivalent to that defined in *Equation 4*.

Equation 56 is one of the fundamental equations in SPME and allows us to calculate the potential mass of analyte extracted by SPME from a headspace sample.

We can make a comparison between the potential extraction efficiency of a compound by SPME against that of HS Trap by dividing *Equation 56* by *Equation 50* as shown in *Equation 57*. Note that $K = K_{SG}$ and $\beta = \beta_{SG}$ in this instance.

$$\text{Relative Extraction} = \frac{M_F}{M_G} = \frac{C_0 \cdot \frac{KFG \cdot VF}{[KSG \cdot \beta SG]}}{C_0 \cdot \frac{VG}{[K+\beta]}} = K_{FG} \cdot \beta_{FG} \quad \text{Equation 57}$$

Where:

β_{FG} is the ratio of the volumes of the two phases (VF/VS)

Equation 57 reveals one of the disadvantages of SPME for headspace sampling – the value of β_{FG} is going to be extremely small because of the very low effective volume of the fiber coating, ***VF.***

The volume of the fiber coating may be calculated using *Equation 58*.

$$V_F = 4 \cdot \pi \cdot D_F \cdot d_F \cdot L_F \quad \text{Equation 58}$$

Where:

D_F is the diameter of the SPME fiber

d_F is the thickness of the coating on the SPME fiber

L_F is the length of the coating on the SPME fiber exposed to the headspace vapor

For a typical SPME system, the fiber diameter, D_F, could be 0.20 mm, the coating thickness, df, 50 µm and the exposed fiber length, L_F, 1.0 cm.

In this case V_F would be 4 x 3.142 x 0.020 x 0.0050 x 1.0 = 0.0013 mL

For a 5 mL sample in a 22 mL vial, the headspace volume, ***VG,*** would be 12 mL and so the value of the phase ratio, β_{FG}, would be 0.0013/12 = 0.00011

Substituting this result into *Equation 57* would indicate that, to get a similar recovery to HS Trap, a fiber coating that has a partition coefficient with the headspace of 1/0.00011 = ~9500 for all analytes will be required. This is going to be very difficult to attain for many compounds.

For this reason, many SPME fibers use highly retentive solid phase coatings in which particles of polymer or carbon-based adsorbents are bonded onto the fiber. Even so, SPME is still going to retain only a small fraction of the total compound present in the headspace and in the original sample.

The other potential issue with SPME concerns the kinetics in achieving equilibration. The presence of the additional phase will add to the equilibration time. Fortunately molecular diffusion in the headspace phase is fast and the thickness of the coating on the fiber is thin – both of these will help accelerate this equilibration process. The area of this phase interface, however, is very low which will slow it down.

One of the difficulties in SPME is that overlapped thermostatting is not possible with the fiber in the vial. The samples must first be thermostatted for a period of time to achieve equilibration between sample and the headspace phase and then an additional equilibration period would be required once the fiber is inserted into the sample vial. This additional step significantly reduces sample throughput.

To improve sample throughput, many SPME methods, do not wait for the system to achieve equilibration but rather sample the headspace for a fixed period which occurs in advance of equilibrium. The analytical performance now becomes much more dependent on the kinetics associated with the various molecules as they transition between the phases rather than their final concentrations defined by thermodynamics. While this approach may reduce the extraction efficiency of the system, it may improve the selectivity of the extraction process significantly – larger molecules will be much slower in moving into the headspace phase. This is, perhaps, the main benefit of using SPME to sample the headspace – much cleaner chromatography. Of course, this technique is going to be most suitable to certain types of sample – those in which the compounds of interest migrate first into the headspace and sorb onto the fiber coating.

Regarding detection limits, typical extracts injected into the GC column will be in the range of 0.1 to 1% of the compounds in the original sample. Thus SPME will offer a slight improvement in detection limits over conventional (equilibrium) headspace but will not approach the detection limits offered by dynamic headspace or HS trap.

Conclusion

Although it is now over 40 years old, headspace-gas chromatography continues to be a very powerful analytical tool. There are many methods in use today based on this technique and more continue to be developed.

This document was designed to assist with the understanding of some of the fundamental relationships involved in HS sampling so that a user can develop better methods to get better and faster data.

If you wish to comment on this document or have suggestions for improving, it please email the author at andrew.tipler@perkinelmer.com.

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Glossary

Activity Coefficient
A factor applied to a partition coefficient to compensate for inter-molecular interactions occurring in high concentration mixtures.

Analyte
A compound of analytical interest

Distribution Ratio or Coefficient
See Partition Coefficient

Dynamic Headspace Sampling
A technique to improve analytical detection limits by sweeping the total headspace vapor from a vial into some form of trap for focusing and subsequent desorption into the GC column.

Equilibrium Headspace Sampling
An analytical technique that thermally equilibrates a sample sealed in a vial and then withdraws a fixed volume of the headspace vapor and injects it into a GC column.

Gas-Tight Syringe Injection
A headspace sampling technique that uses a gas-tight syringe to sample and inject headspace vapor into a GC column.

Headspace
The vapor that resides above a sample in a sealed vial

Headspace Trap
A technique to improve detection limits by allowing the headspace vapor in a pressurized vial to be fully released through some form of trap for focusing and subsequent desorption into the GC column. Multiple cycles may be performed to effectively transfer the total vapor to the GC column.

Multiple Headspace Extraction (MHE)
A multiple extraction technique used to predict total analyte content in solid or heterogeneous samples

Partial Pressure
The pressure each component in a mixture contributes towards the total pressure of that mixture.

Partition Ratio or Coefficient
A thermodynamic property that will define the relative concentrations of a compound between two phases – in this case the sample and the headspace vapor.

Phase Ratio
The ratio of the volume of the headspace phase to the volume of the sample phase

Pressure Balanced Sampling
A gated headspace injection technique that releases pressurized headspace vapor directly into a GC column or transfer line.

Purge and Trap
A sampling technique that is largely used in water analysis in which a stream of carrier gas is bubbled through the sample and into an adsorbent trap. The collected VOCs are thermally desorbed into the GC column for analysis.

Static Headspace Sampling
See Equilibrium Headspace Sampling

Total Vaporization Technique (TVT)
A technique in which a small amount of sample is totally evaporated inside a sealed vial

Valve Loop Sampling
A headspace sampling technique that uses a gas sampling valve loop to sample and inject headspace vapor into a GC column.

Vapor Pressure (VP)
The pressure asserted by a compound at a given temperature

Volatile Organic Compounds (VOCs)
Various definitions exist but generally this refers to organic compounds that have boiling points up to 250 °C and are of analytical interest. Headspace analysis is really only applicable to VOCs.

Zero Dilution Liner (ZDL)
A special GC injector liner that allows the injector to serve as an interface between a Pressure Balanced HS sampler and GC column without dilution of the sample

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