

A Technical Guide for Static Headspace Analysis Using GC



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Static headspace gas chromatography (GC) is a technique used for the concentration and analysis of volatile organic compounds. This technique is relatively simple and can provide sensitivity similar to dynamic purge and trap analysis. The popularity of this technique has grown and has gained worldwide acceptance for analyses of alcohols in blood and residual solvents in pharmaceutical products. Other common applications include industrial analyses of monomers in polymers and plastic, flavor compounds in beverages and food products, and fragrances in perfumes and cosmetics.

Sample matrices like blood, plastic, and cosmetics contain high molecular weight, non-volatile material that can remain in the GC system and result in poor analytical performance. Many laboratory analysts use extensive sample preparation techniques to extract and concentrate the compounds of interest from this unwanted non-volatile material. These extraction and concentration techniques can become time consuming and costly. Static headspace analysis avoids this time and cost by directly sampling the volatile headspace from the container in which the sample is placed.

Because of the diversities in the industry and related products, this guide attempts to cover only the basic principles of static headspace and demonstrate how to apply them to achieve optimum chromatographic results. With an understanding of these principles, various instrumentation will then be reviewed to help build upon this knowledge and identify the benefits and potential problems associated with each mode of sample transfer. Information from the *Basic Principles* and *Instrumentation* sections of this guide can then be brought together and applied to the conditions and methodology of common analyses. Like most applications, a variety of problems may arise in which the *System Optimization* section will help to identify these problems and offer techniques to help resolve them.

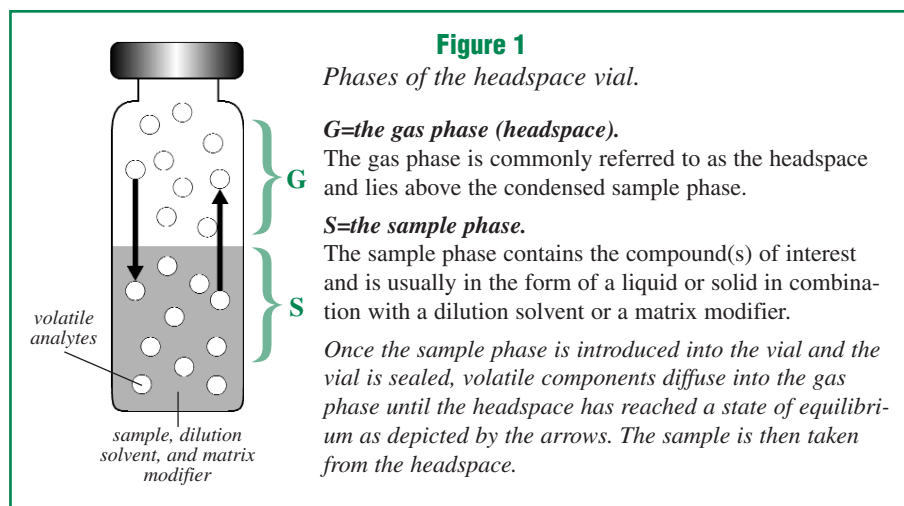
Time and money are two of the many reasons why an analyst would use static headspace analysis. Other reasons may include ease of operation and the ability to assay a variety of sample matrices.



Basic Principles of Headspace Analysis

Most consumer products and biological samples are composed of a wide variety of compounds that differ in molecular weight, polarity, and volatility. For complex samples like these, headspace sampling is the fastest and cleanest method for analyzing volatile organic compounds. A headspace sample is normally prepared in a vial containing the sample, the dilution solvent, a matrix modifier, and the headspace (see **Figure 1**). Volatile components from complex sample mixtures can be extracted from non-volatile sample components and isolated in the headspace or vapor portion of a sample vial. An aliquot of the vapor in the headspace is delivered to a GC system for separation of all of the volatile components.

In order to achieve the best performance when using headspace/GC, careful attention should be used in sample preparation and instrument setup. Key issues to address when setting up headspace/GC systems include minimizing system dead volume, maintaining inert sample flow paths, and achieving efficient sample transfer. These issues, as well as other instrument setup-related topics, are addressed later in the *System Optimization* section of this guide.



Partition Coefficient

Samples must be prepared to maximize the concentration of the volatile components in the headspace, and minimize unwanted contamination from other compounds in the sample matrix. To help determine the concentration of an analyte in the headspace, you will need to calculate the partition coefficient (K), which is defined as the equilibrium distribution of an analyte between the sample phase and the gas phase (**Figure 2**).

Compounds that have low K values will tend to partition more readily into the gas phase, and have relatively high responses and low limits of detection (**Figure 3**). An example of this would be hexane in water: at 40°C, hexane has a K value of 0.14 in an air-water system. Compounds that have high K values will tend to partition less readily into the gas phase and have relatively low response and high limits of detection. An example of this would be ethanol in water: at 40°C, ethanol has a K value of 1355 in an air-water system. Partition coefficient values for other common compounds are shown in **Table I**.

Figure 2
K and β are important variables in headspace analysis.

Equation 1
Partition Coefficient (K) = C_s/C_g

Equation 2
Phase Ratio (β) = V_g/V_s

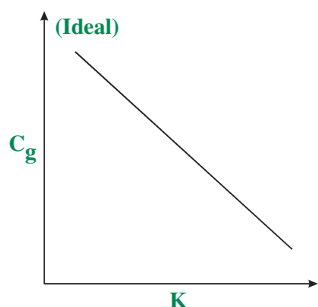
C_s =concentration of analyte in sample phase
 C_g =concentration of analyte in gas phase
 V_s =volume of sample phase
 V_g =volume of gas phase

Table I
K Values of Common Solvents in Air-Water Systems at 40°C

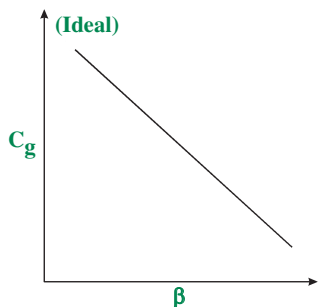
Solvent	K Value
cyclohexane	0.077
n-hexane	0.14
tetrachloroethylene	1.48
1,1,1-trichloromethane	1.65
o-xylene	2.44
toluene	2.82
benzene	2.90
dichloromethane	5.65
n-butyl acetate	31.4
ethyl acetate	62.4
methyl ethyl ketone	139.5
n-butanol	647
isopropanol	825
ethanol	1355
dioxane	1618

Figure 3

Sensitivity is increased when K is minimized.

**Figure 4**

Sensitivity is increased when β is minimized.



K can be lowered by changing the temperature at which the vial is equilibrated or by changing the composition of the sample matrix. In the case of ethanol, K can be lowered from 1355 to 328 by raising the temperature of the vial from 40°C to 80°C. It can be lowered even further by introducing inorganic salt into the aqueous sample matrix. High salt concentrations in aqueous samples decrease the solubility of polar organic volatiles in the sample matrix and promote their transfer into the headspace, resulting in lower K values. However, the magnitude of the salting-out effect on K is not the same for all compounds. Compounds with K values that are already relatively low will experience very little change in partition coefficient after adding a salt to an aqueous sample matrix. Generally, volatile polar compounds in polar matrices (aqueous samples) will experience the largest shifts in K and have higher responses after the addition of salt to the sample matrix. **Table II** lists most of the common salts used for salting-out procedures.

Table II

Common salts used to decrease matrix effects.

ammonium chloride
ammonium sulfate
sodium chloride
sodium citrate
sodium sulfate
potassium carbonate

Phase Ratio

The phase ratio (β) is defined as the relative volume of the headspace compared to volume of the sample in the sample vial (**Figure 2**). Lower values for β (i.e., larger sample size) will yield higher responses for volatile compounds (**Figure 4**). However, decreasing the β value will not always yield the increase in response needed to improve sensitivity. When β is decreased by increasing the sample size, compounds with high K values partition less into the headspace compared to compounds with low K values, and yield correspondingly smaller changes in C_g .

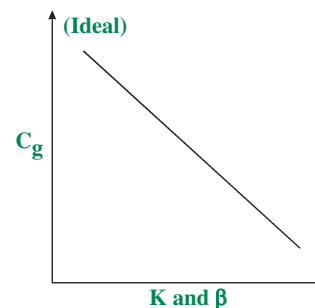
Samples that contain compounds with high K values need to be optimized to provide the lowest K value before changes are made in the phase ratio.

Combining K and β

Partition coefficients and phase ratios work together to determine the final concentration of volatile compounds in the headspace of sample vials. The concentration of volatile compounds in the gas phase can be expressed as $C_g = C_o / (K + \beta)$ (where C_g is the concentration of volatile analytes in the gas phase and C_o is the original concentration of volatile analytes in the sample). Striving for the lowest values for both K and β will result in higher concentrations of volatile analytes in the gas phase and, therefore, better sensitivity (**Figure 5**).

Figure 5

Lower K and β result in higher C_g and better sensitivity.



Derivatization/Reaction Headspace

Derivatization is another technique that can be used to increase sensitivity and chromatographic performance for specific compounds. Compounds such as acids, alcohols, and amines are difficult to analyze because of the presence of reactive hydrogens. When attempting to analyze these types of compounds, they can react with the surface of the injection port or the analytical column and result in tailing peaks and low response. In addition, they may be highly soluble in the sample phase, causing very poor partitioning into the headspace and low response. Derivatization can improve their volatility, as well as reduce the potential for surface adsorption once they enter the GC system.

Common derivatization techniques used in reaction headspace/GC are esterification, acetylation, silylation, and alkylation. Any of these derivatization techniques can be performed using the sample vial as the reaction vessel (see **Table III** for a list of commonly used reagents). Although derivatization may improve chromatographic performance and volatility for some compounds, derivatization reactions may introduce other problems into the analytical scheme. Derivatization reagents as well as the by-products from derivatization reaction may be volatile and can partition into the headspace along with derivatized compounds. These extra volatile compounds may pose problems by eluting with similar retention times as the compounds of interest, causing either partial or complete coelutions.

Derivatization reactions also are typically run at elevated temperatures. Pressures inside the sample vial may exceed the pressure handling capabilities of the vial or the septa. Specially designed septa are available that allow excess pressure to be vented during derivatization reactions.

Table III

Common reagents used to derivatize compounds of interest.

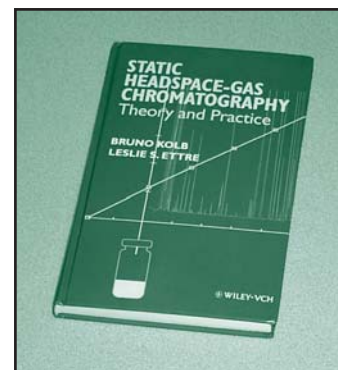
Compound of Interest	Derivatizing Reagent	Resulting Derivative
fatty acids	methanol with boron trifluoride	esterification
glycerol	acetic anhydride with sodium carbonate	acetylation

For more information on derivatization, please refer to the "Handbook of Analytical Derivatization Reactions" by Daniel R. Knapp or to the text at right.

Headspace Sample Size

In addition to working with K , β , and derivatization reactions, sensitivity also can be improved by simply increasing the size of the headspace sample that is withdrawn from the sample vial and transferred to the GC. Increasing the sample size also means that the amount of time it takes to transfer the sample to the column will increase in proportion to the column volumetric flow rate. Sample size can be increased only to the point that increases in peak width, as a result of longer sample transfer times, will not affect chromatographic separations. Larger sample sizes and longer transfer times can be offset by using cryogenic cooling and sample refocusing at the head of the column.

For more information on headspace analysis, check out the textbook, **Static Headspace-Gas Chromatography, Theory and Practice** by Bruno Kolb and Leslie S. Ettre.



System Optimization (Troubleshooting)

Chromatographic performance in Headspace/GC is greatly influenced by how the sample is introduced into the analytical column. Variables that affect sample preparation and transfer of the sample from the headspace unit to the analytical column must be optimized to obtain reproducible and efficient separations. Key issues to address when setting up headspace/GC systems include minimizing system dead volume, maintaining inert sample flow paths, and achieving efficient sample transfer. This section will explain how to optimize areas that are critical in addressing these issues and providing good chromatographic performance.

Sample Preparation

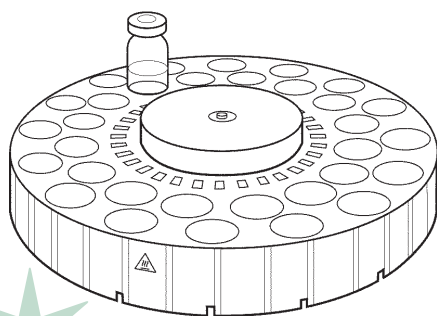
Samples for headspace/GC must be prepared in such a manner as to maximize the concentration of the volatile sample components in the headspace while minimizing unwanted contamination from other compounds in the sample matrix. Sample matrices such as biological samples, plastics, and cosmetics can contain high molecular weight, volatile material that can be transferred to the GC system. Water from the sample matrix also can cause problems by recondensing in the transfer line. Incomplete or inefficient transfer of high molecular weight compounds or water vapor from sample matrices can produce adsorptive areas in the transfer line or injection port that can lead to split peaks, tailing peaks, or irreproducible responses or retention times. To minimize matrix problems and prevent water condensation from aqueous samples, use a higher transfer line temperature (~125°C–150°C).

High-concentration samples need to be prepared appropriately to obtain optimal chromatography. High-concentration samples can produce ghost peaks in subsequent analyses due to carryover of sample from previous injections. Sample carryover can be minimized by using higher transfer line and injection port temperatures, but some samples may need to be diluted and re-analyzed to obtain reliable results. Additionally, we recommend injecting standards and samples in order from low to high concentrations to help minimize carryover. When sample carryover or ghost peaks are evident, you may need to bake-out the column at its maximum operating temperature and elevate the transfer line temperature in order to remove all of the residual sample. If high-concentration samples are anticipated in a sequence of samples, running a blank after the suspected samples will reduce carryover contamination of following ones. It is good lab practice to handle standards and method blanks the same way samples are handled to make any vial or sample preparation problems easier to identify.

Sample Vial

Sample vials should be selected to match the type and size of the sample being analyzed. Always use pre-cleaned vials for sample preparation and storage. Vials that are not properly cleaned prior to packaging or that absorb contaminants during shipping can produce unknown chromatographic peaks, or “ghost peaks.” Ghost peaks that are the result of vial contamination can be identified by running method blanks and zero standards during the system calibration sequence.

The septa used to seal the headspace vials also can be a source for contaminants, which can bleed into the headspace of the vial during equilibration. These contaminants can appear as single peaks or multiple peak patterns. Some septa are available with a Teflon[®] face to eliminate bleed from the rubber portion of the septa. These septa should not be re-used. Once the Teflon[®] face has been punctured by a syringe, contaminants from the rubber portion of the septa can migrate into the headspace and show up as unidentified peaks. Again, the use of method blanks can help to determine the source of contaminants.

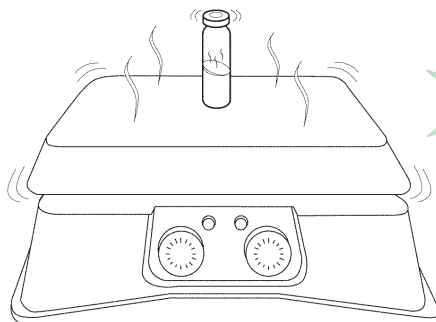


Always use pre-cleaned vials for sample preparation and storage.

Sample Vial Heater and Mixer

Once the sample is placed inside a clean, non-contaminating vial and the vial is sealed, volatile compounds from the sample will partition into the headspace until a state of equilibrium is reached. The rate at which volatile compounds partition out of the sample matrix and into the headspace, as well as the equilibrium concentration of volatile compounds in the headspace depends on several parameters (see also *Introduction* of this guide).

Temperature, time, and mixing can be used to improve the transfer of volatile analytes from the sample into the headspace of the vial. Adjusting the temperature of the sample will change the solubility of the analyte in the sample matrix and can be used to drive the equilibrium in favor of the gas phase. Sufficient time must be built into the sample cycle in order to achieve a constant state of equilibrium. Some sample matrices require longer equilibration times due to physical characteristics like high viscosity. Shaking or vibrating the vial during heating can assist in achieving equilibrium more quickly by exposing more sample surface area for the transfer of volatile analytes to the headspace.



Shaking or vibrating the vial during heating can assist in achieving equilibrium.



Sampling

There are several techniques used to transfer samples from the vial to the GC. When using a *gas-tight syringe* for sampling, heat the syringe to a temperature comparable to the sample vial temperature. This minimizes pressure differences and condensation problems. To prevent carryover from inside the syringe, flush the syringe after each injection. Because gas-tight syringe samplers inject through the GC injection port septum, ensure the septum is well maintained to decrease the possibility of a leak.

For *balanced-pressure sampling* instruments, analysts should consider the inertness and efficiency of the components that make up the sample pathway inside the autosampler. If sensitive compounds are being analyzed, an inert pathway should be used to decrease possible adsorption. Materials such as stainless steel, nickel, Silcosteel[®] and Teflon[®] coatings, or KEL-F[®] parts can be used to minimize sample adsorption and peak tailing. Transfer line internal diameter should be as narrow as possible to help maintain narrow sample band widths and symmetrical peak shapes (see the following optimization of transfer lines for more information). Analysts also should ensure that balanced-pressure instruments are leak-free and operate with the least amount of dead volume in the sample flow path. This will help obtain optimal peak shape and sensitivity.

When using *pressure-loop sampling* instruments, the same concerns apply as with gas-tight syringe and balanced-pressure systems. Inert sample pathways and low dead volume systems will yield the best chromatographic performance. In pressure-loop systems, a gas sampling valve with a sample loop is used to transfer the sample from the headspace unit to the GC. Adequate purging of the sample valve and loop will guard against sample carryover. If low response or broad peaks are observed, it may be necessary to increase the sample vial pressure to ensure that the sample loop is being completely filled with headspace sample. If there are extraneous peaks present due to carryover of matrix contaminants, increase the sample valve temperature to prevent sample carryover, condensation, and contamination.